Multi-year Demography and Ecological Genetics of the Common Plant *Lychnis flos-cuculi* in a Fragmented Landscape

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Prof. Dr. Bernhard Schmid (Vorsitz)
Prof. Dr. Markus Fischer (Leitung der Dissertation)
Prof. Dr. Christine Müller
Prof. Dr. Nicolas Perrin

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Contents

Les hommes de chez toi cultivent cinq mille roses dans un même jardin… et ils n'y trouvent pas ce qu'ils cherchent…
Les yeux sont aveugles, il faut chercher avec le coeur…

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Chapter 1

General Introduction

Motivation

This has both a conservation biological motivation and one of ecological genetics. The main aim was to address advanced questions on the consequences of landscape fragmentation for a common plant species in Switzerland. For several reasons it is important to consider that, not only rare plant species are affected by modern landscape fragmentation, but also common species. Actually, a decline of common plant species caused by fragmentation may largely influence the whole ecosystem and this also affect other species. Moreover, it is essential to also consider the longer-term future and to realise that some common species of today may become the rare and endangered species of tomorrow. This led me to study the consequences of habitat fragmentation for the common plant species, *Lychnis flos-cuculi*. Moreover, because most previous knowledge on effects of habitat fragmentation is based on short-term studies I monitored individual plant fitness, population demography, and consequences of inbreeding and outbreeding for plant fitness for several years.

In this first chapter, I introduce the scientific background upon which hypotheses and experiments are based.

Context

The background

In the course of the history of the earth, biodiversity encountered different extinction peaks, characterized by the extinction of more than one geographically wide-spread higher taxon during a relatively short time interval (Bambach 2006). The two last recent centuries experienced the highest species extinction rate ever recorded. Obviously, the cause of this last extinction wave is human activity (Tilman and Lehman 2001). Modern development of industrial activities and demographic economic expansion present an increasing pressure on the natural environment and result in the conversion of landscapes including the loss and fragmentation of habitats.

In fragmented landscapes, the size of habitats and their connectivity are reduced. This has consequences at several levels of biological organisation; including the ecosystem, species, population and genetic levels. Genetic diversity plays an important role in the persistence of species and populations. Genetic variation of populations is essential and has to be maintained at a high level to face future environmental changes and long term pressure of natural selection (Ballou and Lacy 1995). Nevertheless, if the available habitat remnants are too small and/or not well enough connected, populations cannot maintain themselves in the long run. Moreover, if the environment is inadequate to maintain populations of many species any more, even currently abundant species may become threatened in the long term (Hanski 2005).

In this thesis, I investigated effects of landscape fragmentation on the common but already declining perennial species *Lychnis flos-cuculi*. My project was part of a bigger project that lasted 6 years (Galeuchet 2003; Perret 2003; Vergnerie 2006). I combined several complementary approaches to assess plant fitness and population demography in many field populations for several years, and longer-term fitness effects of experimental inbreeding and outbreeding within and between populations in a controlled environment.

The problem

Habitat fragmentation

Survival and growth of natural plant populations depend on the availability of suitable habitats (Lienert 2004). Moreover, populations have to maintain a minimal population size to maintain plant fitness at a level allowing for long-term persistence (Gibbs 2001, Reed 2005). However, modern agriculture and urbanization have changed natural land use and distribution for the past 100 years. The landscape has been fragmented and habitats were divided into smaller and less suitable areas for natural species. It is now known that fragmentation threatens the survival of many species because populations become smaller (Young et al. 1996). Moreover, fragmentation splits the natural structure of the landscape matrix into disjoints patches, which affects many biological interactions (Tscharntke and Brandl 2004). Moreover, if connectivity between populations is drastically reduced, gene flow is reduced accordingly, which leads to a decrease of genetic diversity and to negative consequences for plant fitness (Gibbs 2001).

The processes

Biotic interactions: Pollinators, Herbivores, and Pathogens

Sexual reproduction of most flowering strongly depends on pollinators. Therefore, pollinators are of major importance for ensuring reproductive success. Moreover, they are essential to maintain the genetic structure of populations and gene flow (Berge et al. 1998). However, the behavior of pollinators may change in response to landscape changes (Goverde et al. 2002, Cresswell and Osborne 2004, Morgan and Scacco 2006).

Plant fitness may also be reduced due to the presence of herbivores and pathogens (Leimu and Koricheva 2006). Herbivores and pathogens attack plant leaves and flowers and generate damages on plants. This reduces fitness directly, and they may cause additional energetic costs either for inducing defenses or for additional growth to compensate the loss (Fletcher et al. 2001, Tscharntke and Brandl 2004, Steets et al. 2006). If plant fitness is reduced in a fragmented landscape, additional plant damage may have especially strong consequences for plant fitness.

Adaptive capacity

In heterogeneous environments, such as our cultural landscape, plants can encounter very different habitat conditions. Moreover, environmental conditions such as levels of nutrient, light and moisture, can change over years, as a result of local changes of land use or as a result of global change. Plants can respond to spatial and temporal heterogeneity of their habitats either by phenotypic plasticity or by adaptation. Phenotypic plasticity is the fastest answer, when genotypes are able to adjust their phenotypes to new conditions (Schlichting 1986). However, plasticity may not always have evolved, because it is constrained by several costs and limits (DeWitt et al. 1998, Schlichting and Pigliucci 1998). Alternatively, plant populations may adapt to local conditions (Kindell et al. 1996, Joshi et al. 2001). Local adaptation presents the advantages to increase fitness under current conditions. However, it reduces the heritability of adapted traits. This reduces the possibility of adaptive responses to further environmental changes. Moreover, local adaptation may have the applied consequence, that transplanting plants between populations may not be very successful (Van Andel 1998).

Genetic drift and inbreeding depression

Population genetics are affected by landscape and habitat fragmentation through a reduction of gene flow and of population sizes (Ellstrand and Elam 1993). In the fragmented landscape, gene flow may not only be reduced according to increased geographic distance between populations. Rather, pollinator service can be modified non-linearly in fragmented landscapes (Goverde et al. 2002). For example, very small populations may receive very little pollinator visits because they are much less attractive for pollinators than larger ones. In isolated populations, genetic variation may be reduced and fixation of deleterious alleles becomes likely due to genetic drift (Barrett and Kohn 1991). In smaller populations, this mechanism becomes more important due to the small genetic sample size and it leads to a loss of variation which reduces the ability of populations to adapt to the changing environment and increases their susceptibility to pest and disease pressures (Barrett and Kohn 1991).

Smaller population sizes also lead to an increase of inbreeding. Landscape fragmentation leads to a reduction in the number and density of conspecifics (Stephens and Sutherland 1999) and to a reduction of pollinator density, or may change their behavior of visiting plants (Goverde et al. 2002, Vergeer et al. 2003). Consequently, self-pollination rate or pollination among related plants may rapidly increase, leading populations to a higher inbreeding rate (Charlesworth and Charlesworth 1990, Young et al. 1996, Hooftman et al. 2004). The main fitness effect of inbreeding is called inbreeding depression (ID) which is defined as a fitness reduction of selfed offspring compared with outcrossed offspring (Barrett and Kohn 1991). An increased inbreeding rate may be translated into lower plant fitness because of the breakdown of heterozygote advantage in one or several loci or due to the increased expression of deleterious alleles in more homozygous plants (Dudash 1990). In combination, genetic drift, inbreeding, and inbreeding depression are likely to lead to a higher risk of extinction of smaller populations.

Purging

Inbreeding depression may decrease and fitness increase by purging of the genetic inbreeding load by selection against individuals expressing detrimental alleles (Hauser and Loeschcke 1995, Carr and Dudash 1997). This may even increase plant fitness to levels higher than the ones observed in more outbred populations. Populations may purge their genetic load more or less rapidly depending on their size. It is supposed to occur more rapidly in small and more inbred populations (Glémin 2003, Willi and Van Buskirk 2005). Thus, purging of inbreeding load may help smaller, more isolated and more inbred population to ameliorate negative effects of inbreeding depression.

Genetic rescue

Genetic erosion may also be counteracted by enhancing gene flow between populations, which increases genetic diversity by importing new genes into the populations (Ellstrand et al. 1989, Ellstrand 1992, Hauser and Loeschcke 1994). The first generation of inter-populations crosses is often characterized by a heterosis effect, where offspring present a

higher fitness than offspring resulting from within population crosses (Willi and Fischer 2005). Potentially this is particularly important for small, isolated, and inbred populations. However, the benefits of inter-population crosses may disappear after some generations because an increase of fitness in the first generation may be due to reversing inbreeding rather than to increasing genetic diversity (Vilas et al. 2006).

Breakdown of co-adapted gene complexes and of local adaptation

Inter-population crosses may also have negative fitness consequences. On the one hand, crossing plants from different populations may break up co-adapted complexes of genes. On the other hand, among population-crosses may disrupt local adaptation by importing new genes, which are less adapted to the maternal environment. Such negative effects, called outbreeding depression, were found in previous studies (Fischer and Matthies 1998, Bailey and McCauley 2006). The degree of outbreeding depression can be expected to differ between populations in relation to their size and history. Plants in smaller populations with a longer history of inbreeding may have more co-adapted genomes, and hence higher outbreeding depression. At the same time, genetic drift is the main genetic force in smaller populations, which may override selection and thus local adaptation may be reduced, which could reduce outbreeding depression. Possibly reflecting a balance between these two opposing forces, sometimes an intermediate optimum outbreeding distance is found (Waser and Price 1989).

The effects

Population structure

Plant can allocate differently to the different life-history traits of growth, survival and reproduction (Garcia and Ehrlén 2002). In particular, they have to find a trade-off between vegetative and reproductive growth to maximize fitness (Sandvik 2001). Different selection pressures on plants can lead to structured populations with different proportions in the different life cycle stages (Oostermeijer et al. 1992). Typically, healthy stable population structures for populations of perennial plants include a high proportion of young

individuals (Mondragón et al. 2004). Habitat fragmentation may affect population structure in the direction of higher proportions of vegetative individuals if fragmentation negatively affects the life stages of reproduction and seedling establishment.

Population growth and Extinction

Persistent populations of perennial plants are typically characterized by population growth rates above 1 and by limited temporal variation in growth rates between years. However, the processes described above can reduce growth rates especially of small and isolated populations.

Moreover, negative effects of environmental, demographic and genetic factors can act together to continuously reduce plant fitness and to increase extinction risk (Leimu and Koricheva 2006). Previous studies showed that populations of some rare species are at high risks of local extinction (Fischer and Stöcklin 1997, Kéry et al. 2001, Lienert 2004). However, it is of great importance to also consider more common and abundant species, which play a major role in ecosystem functioning.

Study species

My target species is *Lychnis flos-cuculi L. (= Silene flos-cuculi (L.) Clairv.) (Caryophyllaceae)*. It occurs in sunny and moist habitats, such as wet hay meadows or calcareous fens ranging from the plains to montane elevations (Münch 1979). The species is widespread and abundant throughout its distribution range in Europe including Iceland with the exception of the arctic region (Jäger 1977). The species is a polycarpic perennial and can reproduce clonally and sexually. It overwinters as a green rosette, and forms secondary rosettes from auxiliary buds, which replace the maternal rosette, and some plants can grow a reproductive stem with up to 50 flowers. Flowers are protandrous and pollinated by several potential pollinators as Lepidoptera, Diptera and Hymenoptera (Vejsnæs and Høvsgaad 1990) but self-pollination is possible and does occur (Biere et al. 1989, Galeuchet et al. 2005a). Several small herbivores, mainly snails, leaf miners and

larvae of lepidoptera feed on vegetative plant parts of *L. flos-cuculi* (Wirooks and Plassmann 1999).

In Switzerland, 90% of wetlands disappeared during the last century (Broggi and Schlegel 1989) which led to a decrease in size and number of populations and an increase in the degree of isolation among populations of *L. flos-cuculi*. Therefore, this common species is a good model to investigate effects of landscape fragmentation for a long-lived perennial.

Contents of this thesis

After the theoretical introduction in **Chapter 1**, I present, in **Chapter 2**, the effects of environmental conditions and landscape fragmentation on plant fitness and the presence and impact of herbivores and pathogens on plants in relation to environmental conditions and landscape fragmentation. I obtained fitness data for 5 consecutive years and for 26 natural populations of *Lychnis flos-cuculi* differing in size and isolation. Thus, I combined long-term monitoring with a large number of populations, which has hardly been undertaken before. Moreover, I simultaneously investigated the effects of biotic and abiotic factors on the fitness of this common plant.

In **Chapter 3**, I complemented the study of individual fitness of Chapter 2 with a demographic analysis of population performance. Such demographic studies are the more powerful the longer the time period of demographic data and the larger number of populations. I present a five-year demographic study of the same 26 *L. flos-cuculi* populations, where I assessed population sizes and the finite growth rates of populations as indicators of temporal population fluctuation and of growth. Moreover, I compared observed and projected structure of populations in order to estimate their deviance from the stable stage structure. Finally, I simulated population development for a period of 50 years to predict extinction rates and the future development of small and large and of more and less isolated populations.

Furthermore, in **Chapter 4,** I studied effects of longer-term inbreeding on plants, and whether they are affected by landscape fragmentation. In the greenhouse, I experimentally crossed plants of 19 populations and I performed three successive

generations of inbreeding to assess effects of inbreeding depression on the common *L. flos-cuculi*. For each generation, I measured plant fitness at different stages of the life cycle. The three generations of inbreeding allowed me to study whether inbreeding depression is purged under inbreeding, and whether this differs between smaller and larger populations. Compared with the earlier studies of inbreeding depression in fragmented plant species, the experiment involved plants of more populations and more generations.

Finally, in **Chapter 5**, I investigated possible outbreeding depression in *L. flos-cuculi* due to the breakdown of local adaptation or of gene complexes. I performed inter-population crosses for 13 populations, 6 small and 7 large, and produced the F2 and back-crosses. This experiment is complementary to the previous one, as it explores whether artificial gene flow between populations could actually be a valuable conservation and restoration method to overcome negative effects of inbreeding and inbreeding depression for plant fitness.

A. TJÄRBLOMSTER, VISCARIA VULGARIS ROEHL.
B. GÖKBLOMSTER, LYCHNIS FLOS CUCULI L.

Chapter 2

Negative Effects of Landscape Fragmentation on Plant Fitness in 26 Natural Populations of *Lychnis flos-cuculi* in Eastern Switzerland

With Gillianne Bowman,

David Galeuchet and Markus Fischer

Abstract

In fragmented agricultural landscapes, plant performance is influenced by environmental characteristics of the habitat remnants on the one hand and by effects of landscape fragmentation on the other. From 2000 to 2004 we investigated plant performance in 26 populations of *Lychnis flos-cuculi* in northeast Switzerland. At higher altitudes, rosettes were smaller and had fewer leaves, and flowering plants had fewer flowers. In populations at sites with vegetation indicating higher moisture levels plants produced smaller rosettes. Moreover, they produced fewer rosette leaves in populations at sites with vegetation indicating lower light levels. Plant damage by herbivores and pathogens was less likely in populations at higher altitudes. In the extremely dry year 2003 pathogen occurrence increased while the occurrence of herbivory decreased. Lower rates of plant survival in smaller populations indicate an Allee effect. Rates of plant survival were also lower in the more isolated of our studied populations. Reproductive plants were more likely to be affected by fungal pathogens in smaller populations. With few exceptions, effects of population characteristics on plant performance did not differ between study years. We conclude that plant performance in different field populations of *Lychnis flos-cuculi* in Switzerland is not only affected by environmental differences, but to a large degree, via high degrees of population isolation and small population sizes, also by landscape fragmentation.

Introduction

Recent habitat fragmentation may affect plant fitness in several ways. Here, Allee effects are of particular interest, i.e. negative effects of small population size of isolated populations on plant performance (Stephens and Sutherland 1999). In smaller and more isolated populations plant fitness can be reduced due to a number of different mechanisms (Lienert 2004), including increased susceptibility to herbivores or pathogens (Fletcher et al. 2001, Thies et al. 2003, Galeuchet et al. 2005a), reduced pollinator service (Barrett and Kohn 1991), and increased inbreeding and inbreeding depression (Ellstrand 1992, Ellstrand and Elam 1993, Young et al. 1996, Willi et al. 2005).

In fragmented agricultural landscapes, plant performance is not only influenced by landscape fragmentation, but also by environmental characteristics of the remaining habitats (Fischer and Matthies 1998). For species of wet grasslands in the pre-Alps, such as our study species *Lychnis flos-cuculi*, important abiotic factors include altitude, and levels of precipitation, moisture, light, and nutrients (Pauli 1998). In addition to direct measurements, useful information on habitat characteristics can also be derived from the composition of the vegetation (Ellenberg 1979, Pauli 1998). A useful tool is provided by Landolt (Landolt 1977), the ecological indicator values attributed to each plant species of the Swiss flora. The average indicator value of the vegetation of a site provides a set of integrated measures of habitat quality.

Perennial plants in the vegetative or reproductive state may be differently affected by landscape fragmentation, either because their biological interactions are different (Parmesan 2000, Kéry et al. 2001) or because genetic effects become apparent at different stages of the life cycle (Hauser and Loeschcke 1995, Russell 1998, Vergeer et al. 2003). Therefore, plant performance should be assessed both for reproductive and for vegetative plants. Plant fitness is usually estimated by measuring characteristics related to sexual reproduction (Jacquemyn et al. 2002) and plant survival. However, for most perennial species, clonal (asexual) reproduction is a further important component of fitness (Pan and Price 2002, Volis et al. 2004).

We studied components of fitness of vegetative and reproductive plants and effects of biotic interactions in 26 natural wild populations of *Lychnis flos-cuculi* in fens and other wet grasslands in North-Eastern Switzerland. Recent fragmentation of these grasslands

allows us assessing the effects of landscape fragmentation on plant fitness of this still common species. We addressed the following specific questions: 1- How is plant fitness affected by environmental conditions? 2- Does landscape fragmentation affect plant fitness? 3- Is the presence of herbivores and pathogens influenced by environmental conditions and/or landscape fragmentation?

Material & Methods

Study Species

Lychnis flos-cuculi L. (= Silene flos cuculi (L.) Clairv.)(Caryophyllaceae) occurs in sunny and moist habitats, such as wet hay meadows or calcareous fens ranging from the plains to montane elevations (Münch 1979). The species is widespread and abundant throughout its distribution range , which comprises most of Europe including Iceland but excluding arctic regions (Jäger 1977). In Switzerland the loss of wet meadows in the last decades has led to a decrease in size and number of populations and an increase in the degree of isolation among populations of *L. flos-cuculi.*

The species is a polycarpic perennial. It overwinters as a green rosette, and forms secondary rosettes from axillary buds, which replace the maternal rosette. Several small herbivores, mainly snails, leaf miners and larvae of lepidoptera feed on vegetative plant parts of *L. flos-cuculi* (Wirooks and Plassmann 1999). Flowering stems reach heights of 20–90 cm and bear dichasial inflorescences with up to 50 flowers. The protandrous flowers are predominantly outcrossed (Biere 1996) but self-pollination does occur (Galeuchet et al. 2005a). Flowers of *L. flos-cuculi* are visited by several potential pollinator species of Lepidoptera, Diptera and Hymenoptera (Vejsnæs and Høvsgaad 1990). Ripe fruit capsules open at the top and up to 200 seeds are dispersed by vibrations of the stiffened stalk. Seeds are able to germinate immediately after dissemination in autumn. At the soil surface, seeds remain viable for only 30 days. Therefore, to survive until the next spring, seeds have to be buried. After one year in the soil 60% of buried seeds of *L. flos-cuculi* still germinate (Bekker et al. 1998).

Study sites

In May 2000, we selected 26 target populations of *L. flos-cuculi* located in calcareous fens in North-East Switzerland at altitudes between 850 m and 1350 m (Table 1). These populations represent a wide range of population sizes and of degree of geographic isolation to other populations. Between 2000 and 2004 average population sizes ranged from 116 to 43'397 flowering individuals (see below). We defined isolation of populations as distance to the nearest population combined with the type of landscape. We considered a population as more isolated when the distance to the nearest population was at least 300 m of open field or 50 m of forest. Of a total of 26 selected populations, we considered 12 as 'more isolated' and 14 as 'less isolated'.

For each population, we obtained longitude, latitude and altitude from Swiss topographic maps (Swiss map 50, Office fédéral de la Topographie, Bern, Switzerland).
We estimated population size by counting the number of flowering individuals at peak of flowering during June and July in 2000, 2001, 2003 and 2004. For further computation we used log-transformed population size. In 2000, to assess habitat quality, we performed vegetation surveys in two plots of 2 m^2 in each population and estimated the cover of each species of vascular plants following Braun-Blanquet (1964). For each population we calculated the mean ecological indicator values for moisture, light, and nutrients after Landolt (Landolt 1977) weighted by species abundance. Population size was independent of these mean indicator values (Galeuchet et al. 2005b).

A microsatellite study of the studied populations showed that gene diversity after Nei ranges from 0.761 to 0.879 and increases with population size (Galeuchet et al. 2005a). The inbreeding coefficient, F_{is}, ranges from 0.297 to 0.585 with an average of 0.467, indicating a mixed mating system with substantial inbreeding in *L. flos-cuculi* (Table 1). Moreover, in our study populations, the inbreeding coefficient F_{is} was positively correlated with altitude and negatively correlated with annual precipitation and moisture levels.

Table 1: Altitude, canton (AR = Appenzell Ausserrohden, SG = St. Gallen, SZ = Schwyz), mean size, isolation (M = more isolated, L = less isolated), location, mean ecological indicator for levels of light, moisture, nutrients, gene diversity and inbreeding coefficient, F_{is} (from the microsatellite study of Galeuchet et al. 2005b), for the 26 natural *Lychnis flos-cuculi* populations of our study.

Sites	Canton	Altitude (m)	Population size	Isolation	Coordinates		Sample size	Light	Moisture	Nutrient	Gene diversity	Inbreeding coefficient (F_{is})
					E	N						
Seilerzwecken	SZ	1330	116	M	697 355	209 930	24	2.51	3.56	2.82	0.761	0.498
Bühler	AR	940	119	M	751 865	250 165	24	2.91	3.94	2.49	0.793	0.441
Allmeindswald	SG	1080	204	M	732 220	237 525	24	2.99	3.68	2.66	0.812	0.335
Gäbriswald	AR	1175	218	M	753 430	249 915	24	2.48	3.63	2.97	0.798	0.525
Höchi	SG	970	302	M	725 865	234 945	24	2.66	4.12	2.51	0.795	0.448
Hasenried	SG	1171	381	M	740 520	233 400	24	2.53	3.78	2.55	0.806	0.529
Hausmösli 1	AR	940	539	L	740 935	242 245	24	2.92	3.41	2.40	0.829	0.452
Gäbrisseeli	AR	1020	979	L	753 945	249 895	48	2.60	3.61	2.63	0.797	0.510
Janseren	SZ	930	1422	M	691 700	214 785	48	2.56	3.48	2.56	0.774	0.416
Wäni	SZ	922	1604	M	699 245	218 905	48	2.94	3.91	2.49	0.824	0.467
Sulzel	SZ	956	1798	L	703 400	222 400	48	2.73	3.75	2.39	0.788	0.472
Steintal	SG	1040	2266	L	728 320	230 140	48	2.71	4.16	2.41	0.762	0.486
Grabserberg	SG	1335	2414	L	747 735	227 290	48	2.56	3.87	2.30	0.820	0.413
Chellen	SG	940	2740	L	730 475	237 395	48	2.74	4.12	2.13	0.847	0.427
Roblosen 1	SZ	897	3456	L	700 950	223 100	30	2.98	3.56	2.13	0.830	0.469
Hausmösli 2	AR	1200	3676	L	740 790	242 135	24	2.78	3.40	2.25	0.775	0.386
Etteren	SZ	1020	3683	M	697 020	213 180	48	2.85	3.68	2.78	0.879	0.544
Fetzeren	AR	1005	7671	L	735 900	241 015	60	2.73	3.39	2.52	0.819	0.519
Roblosen 2	SZ	900	8364	L	700 760	223 165	24	2.80	3.75	2.49	0.832	0.542
Wideralp (Schwägalp)	SG	1350	8479	L	741 465	234 620	48	2.52	3.94	2.53	0.797	0.297
Stein	SG	950	8562	M	733 655	228 725	48	2.68	3.58	2.60	0.789	0.585
Feusisberg	SZ	865	8824	M	697 570	224 390	48	2.97	3.69	2.86	0.852	0.451
Bannholz	SZ	947	10917	L	704 130	221 425	48	2.87	3.23	2.89	0.811	0.481
Landscheidi	AR	935	11325	M	734 638	240 576	30	2.83	3.19	2.74	0.811	0.316
Feldmoos	SG	890	14739	L	731 175	232 095	48	2.73	3.61	2.59	0.833	0.521
Munzenriet	SG	1030	43397	L	744 845	229 390	48	2.85	3.72	2.47	0.821	0.403

Study plants and measurements

In May 2000, in 15 larger populations we established two blocks of 50 x 50 m at distances of 5 to 135 m from each other. We established only one such block in the 11 smaller populations. In each block, we marked 24 randomly selected *L. flos-cuculi* individuals with labels and established 18 x 18 cm plots around each of the target plants, which we marked with two 30 cm long iron nails stuck into the ground. We monitored the marked plants yearly at flowering time from 2000 to 2004 and classified each plant as vegetative, reproductive, or dead. This allowed us to estimate rates of survival and flowering in each population. As vegetative fitness traits, we recorded the number of rosettes and the diameter of the main rosette of each plant, i.e. for both vegetative and reproductive plants. For plants remaining vegetative we also recorded the number of leaves of the main rosette. For reproductive plants, we further measured the total number of flowers and fruits. To quantify levels of biotic interactions, we recorded the presence or absence of damage on rosette leaves by herbivores or by fungal pathogens.

Data analysis

With the statistical package S-PLUS (Version 6.1/6.2, 2002 Insightful Corp., Seattle, USA) we analysed variation in measures of plant performance, survival, and biotic interactions. We considered six parameters as explanatory population characteristics, altitude above sea level, mean ecological indicator values of levels of light, moisture, and nutrients, population size, and population isolation. Moreover, to also assess the influence of genetic diversity of populations we added gene diversity (GD) and inbreeding coefficient (F_{is}) of the populations. For each year and each population we considered annual precipitation (as obtained from the climate station network of Meteo Schweiz, Swiss Meteorological Database) as further explanatory variable. We analysed our hierarchical data with linear and generalized linear models, where the factor population was considered as error term for population characteristics and the interaction between population and year as error term for populations, year, the covariate precipitation and all the interaction terms. We used linear models and generalized linear models for continuous and binary data, respectively. Because

of their different growth forms we analysed fitness traits separately for vegetative and reproductive plants.

As we observed the same plants in the same 26 populations for 5 consecutive years, we accounted for repeated measures by correcting the degrees of freedom of within-subject factors with the Huynh-Feldt epsilon (Scheiner and Gurevitch 2001) as calculated with the statistical package R (R Development Core Team 2006).

Results

Plant performance in relation to environmental conditions

Vegetative plants produced fewer rosette leaves in populations at higher altitudes and with lower light indicator values (Table 2; Figure 1). Similarly, reproductive plants produced fewer flowers in populations at higher altitude (Table 3; Figure 1). In years with higher precipitation plants produced rosettes of smaller diameter (Table 3; Figure 1). Moreover, the proportion of vegetative plants affected by herbivory was lower in populations at higher altitude and with higher moisture indicator value (Table 4; Figure 1). Similarly, the proportion of plants affected by fungal infection was lower in populations at higher altitude (Table 4).

The proportion of plants affected by herbivory was higher in the years 2000-2002 than in the dry year 2003 and the following year 2004. In contrast, the proportion of plants affected by pathogen infection was lower in the years 2000-2002 than in the dry year 2003 and the following year 2004 (Figure 2).

Table 2: ANOVA table for the fitness measures number of rosettes, rosette diameter and number of rosette leaves of vegetative plants in 26 natural populations of *Lychnis flos-cuculi*. Interactions between population characteristics and year were not significant and are not presented. We present degrees of freedom (df), F, and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The correction factor ε after Huynh and Feldt was 0.687 for number of vegetative rosettes, 1.0126 for rosette diameter, and 0.9879 for number of rosette leaves.

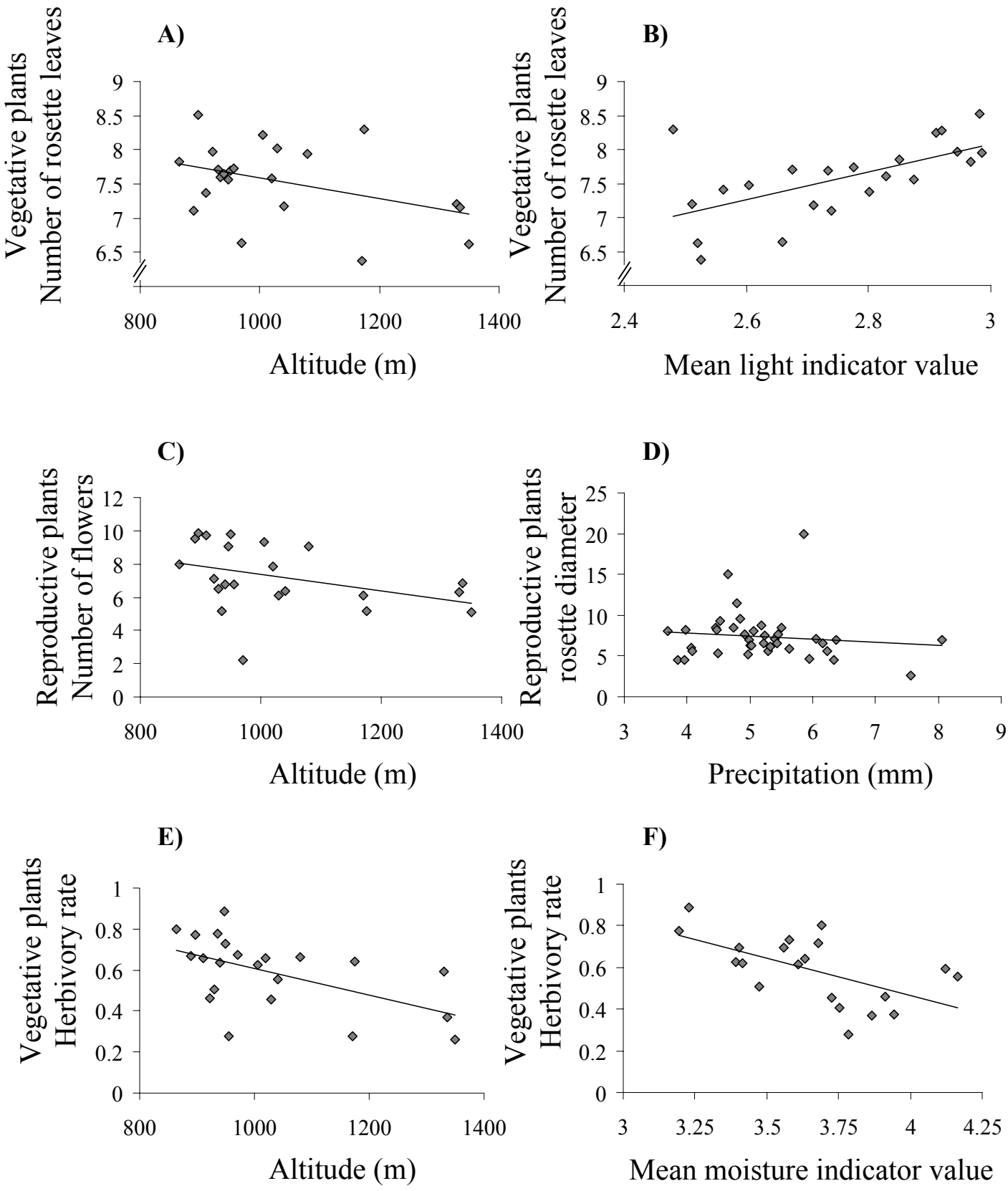
Vegetative plants	**Number of vegetative rosettes**				**Rosette diameter**				**Number of rosette leaves**			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Altitude	1	39.3589	2.7604	0.1139	1	91.9590	0.8985	0.3557	1	133.3725	7.7417	0.0123*
Light	1	9.2148	0.6463	0.4319	1	237.3860	2.3195	0.1451	1	57.8541	3.3582	0.0835+
Moisture	1	14.3712	1.0079	0.3287	1	135.7820	1.3267	0.2645	1	41.8715	2.4304	0.1364
Nutrient	1	3.1546	0.2212	0.6437	1	7.9370	0.0776	0.7838	1	0.2527	0.0147	0.9049
Population size	1	48.3822	3.3932	0.0820+	1	341.2300	3.3341	0.0845+	1	22.8081	1.3239	0.2649
Isolation	1	0.5863	0.0411	0.8416	1	176.0250	1.7199	0.2062	1	6.2955	0.3654	0.5531
Gene diversity	1	1.5822	0.1110	0.7429	1	4.3670	0.0427	0.8387	1	0.1103	0.0064	0.9371
Inbreeding coefficient (F_{is})	1	1.7184	0.1205	0.7325	1	165.1150	1.6133	0.2202	1	32.6477	1.8950	0.1855
Population	18	14.2585	1.5541	0.1108	18	102.3450	4.4668	<0.001***	18	17.2279	1.6412	0.0724+
Year	3	64.7052	7.0523	<0.001***	3	1029.2240	44.9199	<0.001***	3	325.1673	30.9760	<0.001***
Precipitation	1	5.7035	0.6216	0.4342	1	41.8150	1.8250	0.1810	1	12.3491	1.1764	0.2817
Population x Year	49	9.1751	2.9784	<0.001***	48	22.9124	1.7709	0.001***	49	9.9029	2.2509	<0.001***
Residuals	1490	3.0805			1419	12.9380			1487	4.3995		

Table 3: ANOVA table of model analyses for fitness of reproductive plants in 26 natural populations of *Lychnis flos-cuculi*. Fitness was assessed as the number of rosettes, rosette diameter, number of flowers and fruits per plants. Interactions between population characteristics and year were presented when significant. We present degrees of freedom (df), F, and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The correction factor ε after Huynh and Feldt was 0.6076 for number of rosettes, 3.1484 for rosette diameter, 0.6132 for number of flowers per plant, and 0.4844 for number of fruits per plant.

Reproductive plants	**Number of rosettes**				**Rosette diameter**				**Number of flowers per plant**				**Number of fruits per plant**			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Altitude	1	0.0419	0.0092	0.9246	1	17.563	2.0543	0.1689	1	48.793	7.6205	0.0129*	1	6.3431	1.7917	0.1974
Light	1	0.0022	0.0005	0.9825	1	1.1536	0.1349	0.7177	1	14.232	2.2228	0.1533	1	3.8288	1.0815	0.3121
Moisture	1	0.0305	0.0067	0.9356	1	0.0021	0.0002	0.9877	1	10.183	1.5904	0.2234	1	7.8570	2.2194	0.1536
Nutrient	1	3.6314	0.7984	0.3834	1	14.405	1.6848	0.2107	1	0.4321	0.0675	0.7980	1	0.7102	0.2006	0.6596
Population size	1	14.694	3.2304	0.0891+	1	103.31	12.083	0.0027**	1	17.306	2.7029	0.1175	1	6.0218	1.7010	0.2086
Isolation	1	1.8834	0.4141	0.5280	1	12.154	1.4216	0.2486	1	15.090	2.3568	0.1421	1	0.6401	0.1808	0.6757
Gene diversity	1	0.0413	0.0091	0.9251	1	10.021	1.1722	0.2933	1	13.467	2.1033	0.1642	1	12.756	3.6031	0.0738+
Inbreeding coefficient (F_{is})	1	4.8952	1.0762	0.3133	1	1.4701	0.1720	0.6833	1	77.422	12.0918	0.0027**	1	20.389	5.7593	0.0274*
Population	18	4.5486	1.7205	0.0938+	18	8.5495	4.4955	<0.001***	18	6.4028	1.7863	0.1307	18	3.5402	1.5497	0.2215
Year	3	25.982	9.8276	0.0039**	3	14.500	7.6246	<0.001***	3	151.96	42.3973	<0.001***	3	60.867	26.6448	<0.001***
Precipitation	1	33.838	12.799	0.0012**	1	21.782	11.453	<0.001***	1	1.9051	0.5315	0.4772	1	3.5108	1.5369	0.2388
Altitude x Year									3	3.5749	0.9974	0.3338				
Light x Year									3	3.4003	0.9487	0.3455				
Moisture x Year									3	18.063	5.0394	0.0403*				
Nutrient x Year									3	13.048	3.6403	0.0757+				
Population size x Year									3	9.7872	2.7306	0.1192				
Isolation x Year									3	20.599	5.7471	0.0300*				
Gene diversity x Year									3	6.9023	1.9257	0.1855				
Inbreeding coefficient x Year									2	0.1555	0.0434	0.8378				
Residuals	49	2.6438			47	1.9018			25	3.5843			47	6.1539		

Table 4: Generalized linear model (binomial) table for occurrence of herbivory and fungal leaf pathogen infection on vegetative and reproductive plants in 26 natural populations of *Lychnis flos-cuculi*. Herbivory and fungal infection were recorded as presence or absence on 24-48 plants per population. We present degrees of freedom (df), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

	Vegetative plants								Reproductive plants							
	Herbivory				Fungi infection				Herbivory				Fungi infection			
Source of variation	df	Mean Deviance	F-ratios	p	df	Mean Deviance	F-ratios	p	df	Mean Deviance	F	p	df	Mean Deviance	F-ratios	p
Altitude	1	93.493	14.777	0.001***	1	1.7297	0.6802	0.4203	1	7.2487	1.5837	0.2243	1	17.6275	3.9016	0.0638+
Light	1	5.6449	0.8922	0.3574	1	0.0251	0.0099	0.9220	1	6.2367	1.3626	0.2583	1	2.3035	0.5098	0.4844
Moisture	1	29.046	4.5907	0.0461*	1	0.2596	0.1021	0.7530	1	1.7780	0.3885	0.5409	1	0.7669	0.1697	0.6852
Nutrient	1	6.1143	0.9664	0.3386	1	0.6788	0.2669	0.6117	1	7.6843	1.6789	0.2114	1	8.1982	1.8145	0.1947
Population size	1	3.8122	0.6025	0.4477	1	0.8787	0.3455	0.5640	1	0.5958	0.1302	0.7225	1	22.4107	4.9602	0.0389*
Isolation	1	1.0142	0.1603	0.6936	1	0.6834	0.2687	0.6105	1	2.4698	0.5396	0.4721	1	4.6850	1.0369	0.3220
Gene diverstity	1	2.6531	0.4193	0.5255	1	1.9195	0.7548	0.3964	1	0.3503	0.0765	0.7852	1	11.1519	2.4683	0.1336
Inbreeding coefficient	1	6.1511	0.9722	0.3372	1	1.0228	0.4022	0.5339	1	14.2718	3.1182	0.0944+	1	2.3700	0.5246	0.4782
Population	18	6.3272	2.2659	0.0118*	18	2.5430	4.2495	<0.001***	18	4.5770	2.2122	0.0302*	18	4.5181	3.6562	0.0012**
Year	3	66.992	23.991	<0.001***	3	130.85	218.66	<0.001***	3	6.3036	3.0466	0.0458*	3	19.0792	15.4398	<0.001***
Precipitation	1	2.5726	0.9213	0.3418	1	0.4015	0.6709	0.4166	1	1.4901	0.7202	0.4035	1	1.2583	1.0182	0.3219
Altitude x Year	3	3.1545	1.1297	0.3461	3	6.1188	10.225	<0.001***	3	16.1308	7.7963	<0.001***	3	5.2390	4.2396	0.0141*
Light x Year	3	8.2288	2.9469	0.0417*	3	1.5282	2.5537	0.0659+	3	0.7375	0.3564	0.7849	3	3.1475	2.5471	0.0769+
Moisture x Year	3	4.0015	1.4330	0.2442	3	0.3532	0.5902	0.6243	3	0.6817	0.3295	0.8041	3	0.8403	0.6800	0.5720
Nutrient x Year	3	2.7264	0.9764	0.4114	3	0.5044	0.8429	0.4769	3	0.5972	0.2886	0.8332	3	0.0703	0.0569	0.9818
Population size x Year	3	1.3072	0.4681	0.7058	3	1.2022	2.0089	0.1246	3	0.6081	0.2939	0.8294	3	1.5589	1.2616	0.3073
Isolation x Year	3	1.7930	0.6421	0.5915	3	4.0013	6.6864	<0.001***	3	4.0086	1.9374	0.1474	3	1.1107	0.8988	0.4546
Gene diversity x Year	3	1.8982	0.6798	0.5686	3	0.4833	0.8076	0.4956	3	5.5156	2.6658	0.0679+	3	3.2817	2.6557	0.0686+
Inbreeding coefficient x Year	3	0.8088	0.2896	0.8327	3	0.4975	0.8314	0.4829	3	0.4798	0.2319	0.8734	3	0.2031	0.1644	0.9194
Population x Year	50	2.7924	2.0286	<0.001***	50	0.5984	0.9038	0.6649	27	2.0690	1.7809	0.009**	27	1.2357	2.1777	<0.001***
Residuals	1587	1.3765			1587	0.6621			939	1.1618			939	0.5674		


A)
Vegetative plants
Number of rosette leaves
9
8.5
8
7.5
7
6.5
800
1000
1200
1400
Altitude (m)
B)
Vegetative plants
Number of rosette leaves
9
8.5
8
7.5
7
6.5
2.4
2.6
2.8
3
Mean light indicator value
C)
Reproductive plants
Number of flowers
12
10
8
6
4
2
0
800
1000
1200
1400
Altitude (m)
D)
Reproductive plants
rosette diameter
25
20
15
10
5
0
3
4
5
6
7
8
9
Precipitation (mm)
E)
Vegetative plants
Herbivory rate
1
0.8
0.6
0.4
0.2
0
800
1000
1200
1400
Altitude (m)
F)
Vegetative plants
Herbivory rate
1
0.8
0.6
0.4
0.2
0
3
3.25
3.5
3.75
4
4.25
Mean moisture indicator value

◄◄

Figure 1: Vegetative plants of *L. flos-cuculi* produced A) more rosette leaves in populations at lower altitudes (ANOVA; $p<0.05$) and B) in populations with higher levels of light (ANOVA; $p<0.1$) C) Reproductive plants from populations from higher altitude produced less flowers (ANOVA; $p<0.05$) D) In populations with higher precipitation reproductive plants had smaller rosettes (ANOVA; $p<0.001$) E) A lower proportion of plants was affected by herbivory in populations at higher altitude (ANOVA; $p<0.01$) and F) in populations with higher moisture indicator levels (ANOVA; $p<0.05$).

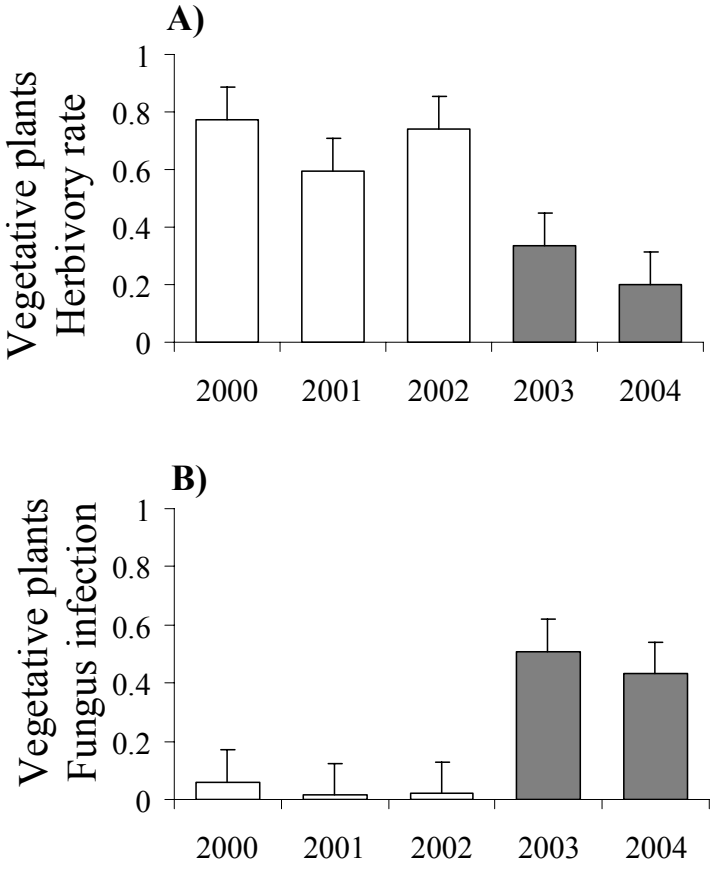


Figure 2: Proportion of plants affected by A) herbivory and B) fungal pathogens in 26 field populations of *Lychnis flos-cuculi* differed between monitoring years (ANOVA; $p<0.001$).

Plant performance in relation to population size, geographic isolation, and inbreeding levels

Plants in smaller populations had lower rates of survival and flowering (Table 5; Figure 3). Moreover, plants from more isolated populations had a higher rate of flowering (Table 5; Figure 3). Finally, reproductive plants had higher vegetative fitness in smaller populations as indicated by more rosettes and larger rosette diameters (Table 3; Figure 4). Fungal plant pathogens were more likely to be found on reproductive plants in smaller populations (Table 4).

These results indicate reduced performance of plants in smaller and more isolated populations. However, in contrast to our hypotheses, plants in populations with higher inbreeding coefficients had more flowers and fruits per plant (Table 4; Figure 5).

Table 5: ANOVA table of model analyses for survival and flowering rate of plants in 26 natural populations of *Lychnis flos-cuculi*. Interactions between population characteristics and year were presented when significant. We present degrees of freedom (df), F, and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The correction factor ε after Huynh and Feldt was 0.7319 for survival, and 0.6134 for flowering rate, respectively.

	Survival rate				Flowering rate			
Source of variation	**df**	**ms**	**F**	**P**	**df**	**ms**	**F**	**P**
Altitude	1	0.0683	1.4275	0.2477	1	0.0066	0.1670	0.6876
Light	1	0.0226	0.4714	0.5011	1	0.0045	0.1142	0.7394
Moisture	1	0.0018	0.0368	0.8500	1	0.0014	0.0369	0.8499
Nutrient	1	0.0034	0.0715	0.7923	1	0.0000	0.0010	0.9752
Population size	1	1.3799	28.8224	<0.001***	1	1.0869	27.6491	<0.001***
Isolation	1	0.1967	4.1080	0.0578^{+}	1	0.3733	9.4964	0.0064**
Gene diversity	1	0.0016	0.0337	0.8565	1	0.0961	2.4435	0.1354
Inbreeding coefficient (Fis)	1	0.0639	1.3340	0.2632	1	0.1352	3.4399	0.0801^{+}
Population	18	0.0479	4.8346	<0.001***	18	0.0393	3.2603	<0.001***
Year	2	1.5388	155.3938	<0.001***	3	1.0109	83.8351	<0.001***
Precipitation	1	0.0014	0.1435	0.7072	1	0.0019	0.1608	0.6902
Residuals	31	0.0099			48	0.0121		

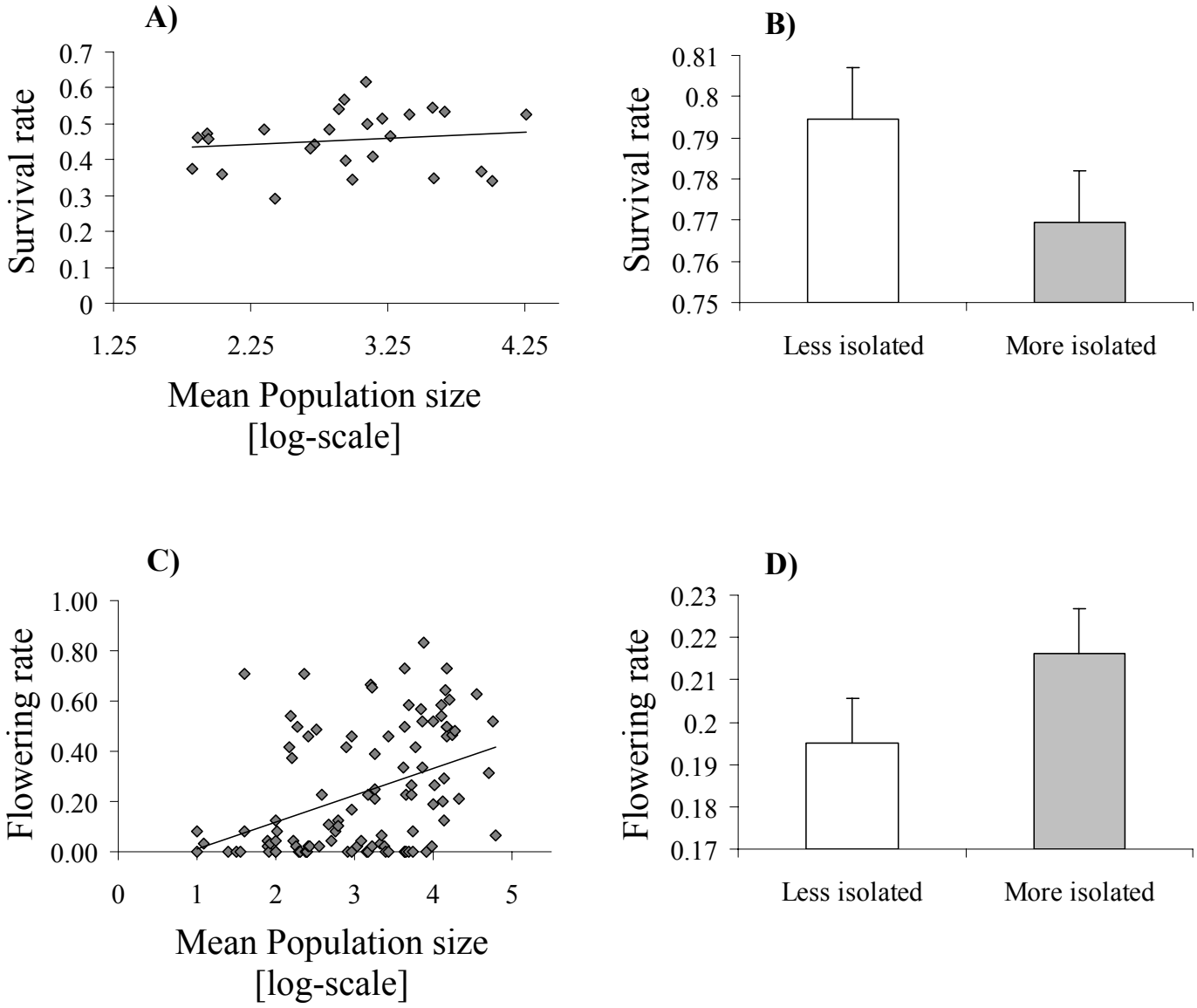


Figure 3: Rates of A) survival and C) flowering rates were smaller in smaller populations of *Lychnis flos-cuculi* (ANOVA model; $p<0.001$). In more isolated populations B) survival rate was lower (ANOVA model; $p=0.057$) and D) flowering rate was higher (ANOVA model; $p<0.01$).

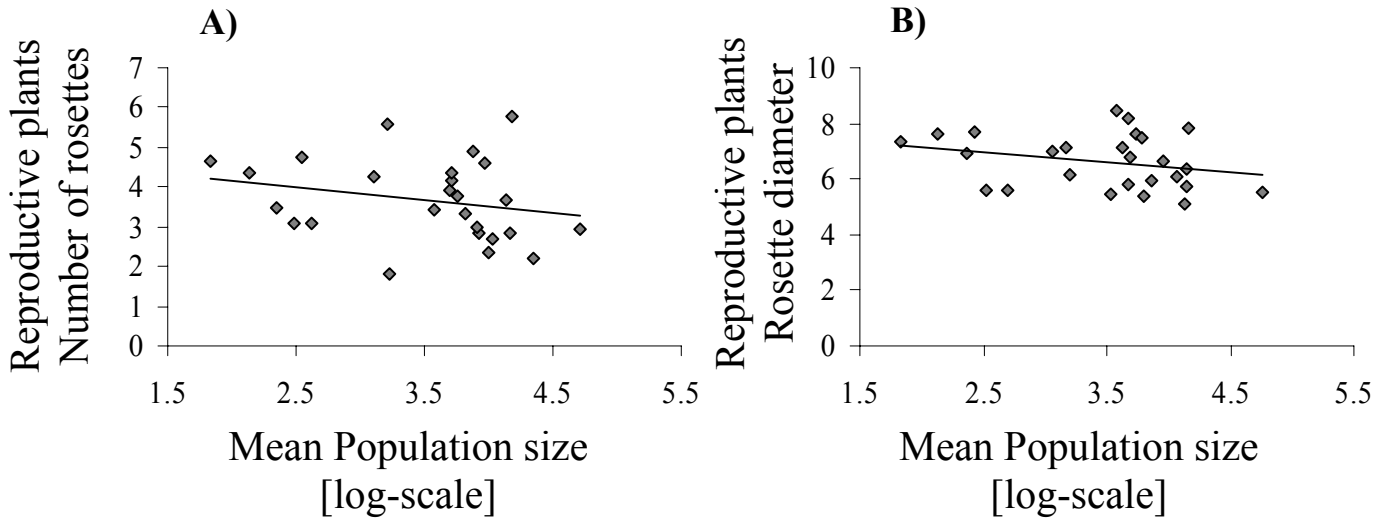


Figure 4: Reproductive plants of *Lychnis flos-cuculi* had A) a higher number of rosettes (ANOVA; $p<0.1$) in smaller populations and B) a larger diameter of the rosettes (ANOVA; $p<0.01$). Therefore, they have a higher vegetative fitness in smaller populations.

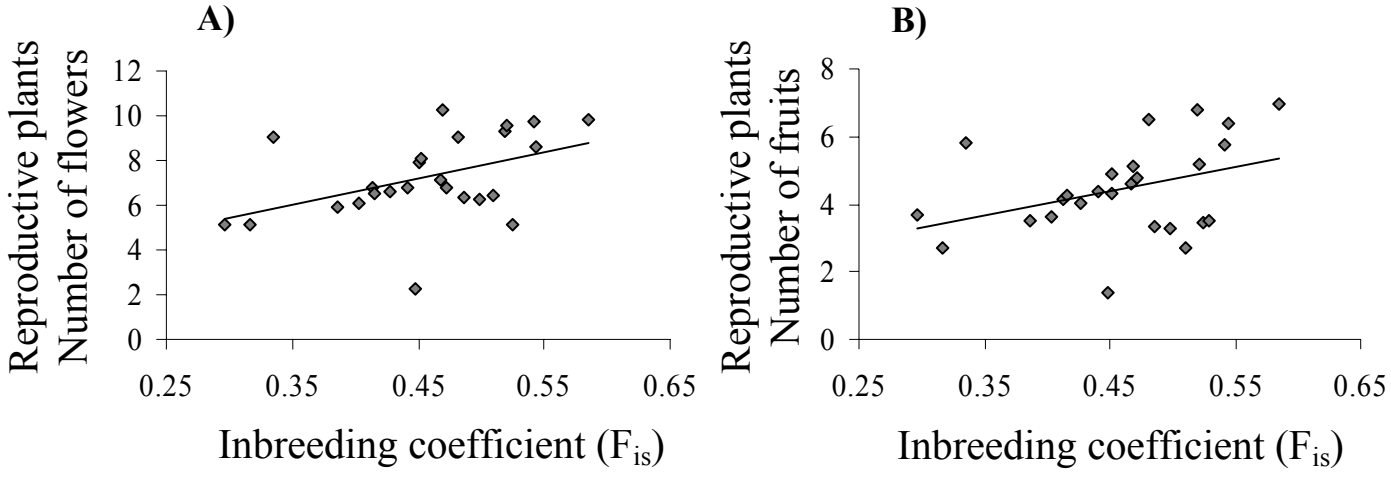


Figure 5: In populations with higher inbreeding coefficient (F_{is}) *Lychnis flos-cuculi* plants had A) more flowers and B) more fruits (ANOVA model; $p<0.05$ for both).

Discussion

Environmental effects on plant performance

Environmental conditions clearly affected the performance of *Lychnis flos-cuculi* plants. Throughout our results, *Lychnis flos-cuculi* had higher fitness at lower altitudes, and at locations with more light, less moisture, and less precipitation. These findings correspond well with the description of the species as a plant of sunny and wet grasslands (Lauber and Wagner 2000), and adds the new information that very moist sites among the grasslands where *L. flos-cuculi* is occurring are less suitable than less moist ones. However, in our study, measures of plant fitness differed between years of observation, which in turn differed in annual precipitation. Clearly, despite the negative effects of too high moisture within years, exceptionally dry conditions such as the ones observed in the summer of 2003, reduce plant fitness in all populations (also see Chapter 3).

Allocation to reproductive and vegetative growth is highly linked to environmental conditions because resources and conditions are usually limiting plant growth in natural environments (Abrahamson 1975). Under favourable conditions clonal growth can be an efficient strategy allowing plants to expand rapidly and to create dense patches of plants within short time (Stöcklin and Winkler 2004). Accordingly, in more favourable environmental conditions *L. flos-cuculi* plants might invest more in clonal growth than in reproduction. Indeed, and in accordance with the findings of a reciprocal replant-transplant experiment with plants of 15 populations of *Lychnis flos-cuculi* plants (Vergnerie 2006), plants grew more vegetative rosettes in environments with higher moisture. Similarly, Schemske showed that reproduction is often reduced in stressful environments (Schemske 1978). Pigliucci and Kolodynska (2002) however, found no significant relationship between environment and the relationship between reproduction and vegetative growth in *Arabidopsis thaliana* (Pigliucci and Kolodynska 2002).

Plant fitness can also be affected by the impact of herbivores (Parmesan 2000, Thies et al. 2003, Steets et al. 2006) and pathogens (Burdon 1993, Schouten et al. 2002) which can depend on environmental conditions. We observed lower rates of plant damage by herbivores and fungal infection in populations at higher altitudes and with higher moisture indicator values.

We conclude that, overall, optimal environmental conditions favouring plant growth and reproduction and minimizing plant damage by herbivores and pathogens for *L. flos-cuculi* are characterised by intermediate moisture value.

Effects of landscape fragmentation on plant performance

Several studies found negative effects of small population size of the population of origin on plant fitness in the common garden (Fischer and Diethart 1998, Fischer et al. 2000, Armbruster and Reed 2005). Usually these effects where explained by increased inbreeding and inbreeding depression in plants from smaller populations, sometimes combined with increased cross-incompatibility and increased mutation load (Fischer et al. 2003, Willi and Fischer 2005, Willi et al. 2005). These studies are very valuable in detecting genetically based Allee effects. However, they do not reveal, whether such effects, even if present, actually matter in the field.

Several studies found performance of plants from smaller populations reduced also in field populations (Oostermeijer et al. 1994, Fischer and Matthies 1998, Hackney and McGraw 2001, Jacquemyn et al. 2002, Lienert and Fischer 2003, Reed 2005). However, many of these studies bear the problem, that they cannot separate the different potential causes underlying such patterns, namely either reduced performance of plants from smaller populations due to small population size, i.e. a true Allee effect, or reduced performance of plants from small populations because of reduced habitat quality, which will eventually also cause small population size. Unfortunately, only very few studies addressed fitness of plants from large and small populations both in the field and in the garden (Fischer and Matthies 1998). Moreover, most studies of plant performance in relation to population size only cover single years.

A previous study with *L. flos-cuculi* showed that plants from smaller field population suffer from reduced numbers of seed per capsule and reduced mass per seed (Galeuchet 2003). Moreover, it showed that plants from small populations grown in the greenhouse perform worse than plants from large populations. However, it remained open, whether such effects are also apparent in other stages of the life cycle in the field. Our 5-year-survey of plant performance revealed further evidence for Allee effects consisting of

reduced survival and reduced flowering rate of plants in smaller populations. Moreover, survival rate was also reduced in populations that are more isolated.

As mentioned above, genetic diversity is reduced, and inbreeding, and inbreeding depression are increased in smaller and more isolated populations (Hansson and Westerberg 2002, Hooftman and Diemer 2003). In our study, however, plants produced more flowers and fruits in populations with higher inbreeding coefficient F_{is}. As the inbreeding coefficient F_{is} was positively correlated with altitude and negatively correlated with annual precipitation and moisture levels (data not shown), higher F_{is} values were measured in, and therefore confounded with, harsher environments. Selection may be stronger in harsher environmental conditions and/or different loci may be expressed differently under different environments (Cheptou 2005). Therefore, we suggest, that selection may have acted more strongly in these populations than in more favourable environments which may have resulted in purging of inbreeding load and therefore reduced inbreeding depression (see also Chapter 4).

Moreover, as described above, smaller and more isolated patches receive fewer pollinators visits (Vejsnæs and Høvsgaad 1990) and patches with plants with larger and higher numbers of inflorescences are more attractive for pollinators (Primack and Kang 1989). Having a higher rate of flowering may constitute an adaptation to counteract reduced pollinator attraction of smaller isolated patches due to the smaller number of flowering individuals (Parmesan 2000, Salomão et al. 2006). Therefore, we suggest addressing the relationship between flowering rate and pollinator visitation rates for small and large populations of *L. flos-cuculi*, which was beyond the scope of the current study.

The presence of herbivores was independent of population size in our study (Table 4). In contrast, other studies found that larger patches attract more herbivores than small patches (Colling and Matthies 2004, Salomão et al. 2006). Possibly our result is the consequence of higher herbivore abundance in larger populations combined with larger plant susceptibility in smaller populations.

In our study, plants in smaller populations rather were infected by fungal leaf pathogens than plants in larger populations. This is in contrast to previous findings of higher infection rates by specialist fungi of *L. flos-cuculi* with the anther smut *Microbotryum violoaceum* (Galeuchet 2003, Perret 2003a) and of *Primula farinosa* with

the smut *Urocystis primulicola* (Lienert 2002). Possibly, the leaf pathogens observed in the present study are generalists, which do not exclusively depend on *Lychnis* as host plant. Such generalist pathogens can well be more prevalent in habitats, which are not very favourable for *Lychnis*. We suggest that more detailed studies are needed to investigate the response of generalist and specialist herbivores and pathogens in fragmented landscapes, and of their effect on the demography of common and rare fragmented plant species.

Conclusion

We observed that environmental condition exert significant influences on plant performance in *L. flos-cuculi* populations. We conclude that optimal environmental conditions to maximize plant growth and fitness and minimize herbivores and pathogens impact are characterized by intermediate moisture levels. In that context, recent and future climate change may play an important role for the persistence of, especially smaller, populations.

We showed that Allee effects are present in the recently fragmented *L. flos-cuculi* populations of Eastern-North of Switzerland. Although it has been suggested that perennial plants with clonal growth may be less affected by recent fragmentation due to their individual long life-spans (Pan and Price 2002, Stöcklin and Winkler 2004), we observed fitness reduction due to small population size and isolation of populations of the still common *Lychnis flos-cuculi*. This clearly shows that not only short-lived and rare, but also long-lived and common species are negatively affected by landscape fragmentation.

Acknowledgments

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Appendix A

Number of *Lychnis flos-cuculi* populations (for survival and flowering), and plants (for all other traits) in each category for the analyses of plant performance of *L. flos-cuculi.*

Survival		Flowering	
year 0		year 0	n = 26
year 1	n = 26	year 1	n = 26
year 2	n = 26	year 2	n = 26
year 3	n = 25	year 3	n = 25
year 4	n = 26	year 4	n = 26
Total	n = 103	Total	n = 129

Reproductive plants

Number of rosettes		Rosette diameter		Number of flowers per plant		Number of fruit per plant		Herbivory and Fungus	
year 0	n = 556	year 0	n = 194	year 0	n = 564	year 0	n = 566	year 0	n = 473
year 1	n = 335	year 1	n = 287	year 1	n = 326	year 1	n = 333	year 1	n = 330
year 2	n = 97	year 2	n = 22	year 2	n = 22	year 2	n = 78	year 2	n = 98
year 3	n = 44	year 3	n = 37	year 3	n = 38	year 3	n = 35	year 3	n = 33
year 4	n = 17	year 4	n = 12	year 4	n = 12	year 4	n = 14	year 4	n = 9
Total	n = 1049	Total	n = 552	Total	n = 962	Total	n = 1026	Total	n = 943

Vegetative plants

Number of rosettes		Rosette diameter		Number of rosette leaves		Herbivory and Fungus	
year 0	n = 442	year 0	n = 408	year 0	n = 445	year 0	n = 423
year 1	n = 522	year 1	n = 502	year 1	n = 506	year 1	n = 508
year 2	n = 543	year 2	n = 531	year 2	n = 539	year 2	n = 541
year 3	n = 392	year 3	n = 367	year 3	n = 392	year 3	n = 391
year 4	n = 253	year 4	n = 250	year 4	n = 251	year 4	n = 253
Total	n = 2152	Total	n = 2058	Total	n = 2133	Total	n = 2116

Chapter 3

A Five-year Demographic Study of Effects of Landscape Fragmentation on 26 Natural Populations of *Lychnis flos-cuculi* in North-Eastern Switzerland

With Gillianne Bowman,

David Galeuchet and Markus Fischer

Abstract

Nowadays, wild plant species are not only influenced by environmental conditions, but also by landscape fragmentation. In this five-year demographic study of 26 natural populations of *Lychnis flos-cuculi*, we show that population sizes were sensitive to environmental conditions and particularly to drought. From projection matrices elaborated from five years of monitoring in each population, we obtained the finite growth rates of populations (λ). Pooled over years, λ was between 1 and 1.04 for all populations, suggesting a quite stable growth of all populations. However, λ varied between years for all populations. Moreover, λ tended to be lower for smaller populations than for larger ones. Due to an excess of older and larger plants, suggesting regressive population structures of *L. flos-cuculi,* observed proportions of plants in different life stages deviated from projected stable stage vectors. Finally, 1000 runs of stochastic matrix-projection simulations over 50 years based on the observed matrices predicted future finite population growth rates λ below 1 for all populations and considerable extinction rates. Moreover, predicted extinction rates were higher for smaller populations than they were for larger populations. Overall, this 5-year demographic study shows, that even the still common *Lychnis flos-cuculi* is negatively affected by landscape fragmentation, which puts especially its small populations at considerable risks of local extinction.

Introduction

With the rapid development of agriculture and urbanization in the last century, our landscapes experienced significant fragmentation leading to a decrease of numbers and sizes of natural habitats for many habitat types, and to a decrease in habitat connectivity (Soons et al. 2005, Noel et al. 2006). Consequently, natural populations of many plant species were also reduced in numbers and size and became more isolated. Clearly, this reduction of available habitat is responsible for the decline of many species (Fahrig 2003, Banks et al. 2005, OFEV 2005).

In remaining populations of sufficiently small size, the so-called Allee effects may take place, i.e. negative effects of small population size of isolated populations on plant performance (Stephens and Sutherland 1999) and on growth rate (Case 2000). Several potential mechanisms underlying such Allee effects were suggested, among them the combined action of genetic drift, inbreeding, and inbreeding depression (Young et al. 1996). Previous short-term demographic studies mainly of rare species suggest that especially small populations are at high risks of local extinction (Fischer and Stöcklin 1997, Kéry et al. 2001, Lienert 2004).

In fragmented agricultural landscapes, plant performance is not only influenced by landscape fragmentation, but also by environmental characteristics of the remaining habitats (Fischer and Matthies 1998). Therefore, negative effects of environmental, demographic and genetic factors can act together to continuously reduce plant fitness and to increase extinction risk (Leimu and Koricheva 2006). Modifications in plant fitness consequently influence population fitness and population demography (Lehtilä et al. in press).

Perennial plants do not necessarily flower every year. Under unfavorable conditions, plants may save energy by reducing investment in reproduction, which represents a large energetic investment for plants. Therefore, refraining from reproduction in one year could result in increased growth and/or reproduction in the following year (Sandvik 2001).

Threatened populations are often characterised by a regressive population structure, were plants of older and larger developmental stages are disproportionately frequent and were recruitment of juveniles is scarce (Oostermeijer et al. 1992, Mondragón et al. 2004).

Persistent populations of perennial plants are typically characterized by population growth rates above 1 and by limited temporal variation in growth rates between years. Therefore, population growth rates below 1 or variation in population growth rates between years may threaten the future of populations (Menges 1992).

Matrix population projection models are powerful tools to explore demographic variation in long-term field-data, to incorporate stochasticity (Mondragón et al. 2004), and to make predictions for the future development of populations, including the predicted stable stage distribution, and predictions of extinction rates (Valverde et al. 2004).

We studied the population demography of 26 natural populations of *Lychnis flos-cuculi* in North-Eastern Switzerland. This species occurs in wet grasslands, which are still common in the study area, but their numbers, sizes, and degrees of connectivity have very much declined during the last century. We monitored population sizes and individual plants in all populations for five years and used these data to parameterise matrix projection models for analysing variation in population growth between populations and years, and to simulate predicted rates of extinction. Specifically, we addressed environmental factors affecting population size variation within and between years. Moreover, we asked whether changes in numbers of flowering plants, in population growth rates and their variation between years, and in projected extinction rates differ between initially smaller and larger populations, and between less and more isolated populations. We also studied whether observed population structures suggested growing or regressive populations.

Material & Methods

Study Species

Lychnis flos-cuculi L. (= *Silene flos-cuculi* (L.) Clairv.*)*(Caryophyllaceae) is a polycarpic perennial. It overwinters as a green rosette, and forms secondary rosettes from axillary buds, which replace the maternal rosette. Flowering stems reach heights of 20–90 cm and bear dichasial inflorescences with up to 50 flowers. The protandrous flowers are predominantly outcrossed (Biere 1996). Flowers of *L. flos-cuculi* are visited by several

potential pollinator species of Lepidoptera, Diptera and Hymenoptera (Vejsnæs and Høvsgaad 1990). Ripe fruit capsules open at the top and up to 200 seeds are dispersed by vibrations of the stiffened stalk. Seeds are able to germinate immediately after dissemination in autumn, but because seeds not germinated after 30 days on soil surface are not viable anymore, seeds have to be buried to survive until next spring. After one year in the soil 60% of seeds of *L. flos-cuculi* germinate, while 20% of seeds are not viable anymore and 20% are lost (Bekker et al. 1998). Several small herbivores, mainly snails, leaf miners and larvae of lepidoptera feed on vegetative plant parts of *L. flos-cuculi* (Wirooks and Plassmann 1999).

The species occurs in sunny and moist habitats, such as wet hay meadows or calcareous fens ranging from the plains to montane elevations (Aeschimann and Burdet 1994). *Lychnis flos-cuculi* is widespread and abundant throughout its distribution range in Europe including Iceland with the exception of the arctic region (Jäger 1977). In Switzerland, the loss of wet meadows in the last decades has led to a decrease in size and number of populations and an increase in the degree of isolation among populations of *L. flos-cuculi*.

Population characteristics

In May 2000, we selected 26 target populations of *L. flos-cuculi* located in calcareous fens in North-East Switzerland at altitudes between 850 m and 1350 m (Table 1). For each population, we obtained longitude, latitude and altitude from Swiss topographic maps (Swiss map 50, Office fédéral de la Topographie, Bern, Switzerland). These populations represent a wide range of population size and of geographic isolation to other populations.

Population sizes were estimated by counting the number of flowering individuals at peak of flowering during June and July in 2000, 2001, 2003 and 2004. Across years, average population sizes ranged from 116 to 43’397 flowering individuals. We considered a population as small when the number of counted plants was lower than 1000. For further computation we used log-transformed population size.

We defined isolation of populations as distance to the nearest population combined with the type of landscape. We considered a population as more isolated when the distance

to the nearest population was more than 300 m of open field or at least 50 m of forest. Of 26 selected populations, 12 were 'more isolated' and 14 'less isolated'.

In 2000, to assess habitat quality, we performed vegetation surveys in two plots of 2 m^2 in each population and estimated the cover of each species of vascular plants following Braun-Blanquet (1964). For each population we calculated the mean ecological indicator values for levels of moisture, light, and nutrients after Landolt (1977) weighted by species abundance. Population size was independent of these mean indicator values (Galeuchet et al. 2005b).

With the statistical package S-PLUS (Version 6.1/6.2, 2002 Insightful Corp. Seattle, USA) we analysed variation in population sizes. We considered four parameters as potential explanatory population characteristics, altitude above sea level, mean indicator values of levels of light, moisture, and nutrients. For each year and each population we considered annual precipitation (as obtained from the climate station network of Meteo Schweiz, Swiss Meteorological Database) as further explanatory variable. We analysed our hierarchical data with ANOVA, where the factor population was considered as error term for population characteristics and the interaction between population and year as error term for populations, year and precipitation.

As we observed the same 26 populations for 5 consecutive years, we accounted for repeated measures by correcting the degrees of freedom of within-subject factors with the Huynh-Feldt epsilon (Scheiner and Gurevitch 2001) as calculated with the statistical package R (R Development Core Team 2006).

Table 1: Characteristics of the 26 study populations of *Lychnis flos-cuculi*. Canton (AR = Appenzell Ausserrohden, SG = St. Gallen, SZ = Schwyz), altitude, mean size, isolation (M=more isolated, L=less isolated), location with coordinates as in Swiss topographical maps, sample size (number of plants), and mean indicator values of the vegetation at the population sites for levels of light, moisture and nutrients.

Sites	Canton	Altitude (m)	Population size	Isolation	Coordinates		Sample size	Light	Moisture	Nutrient
					E	N				
Seilerzwecken	SZ	1330	116	M	697 355	209 930	24	2.51	3.56	2.82
Bühler	AR	940	119	M	751 865	250 165	24	2.91	3.94	2.49
Allmeindswald	SG	1080	204	M	732 220	237 525	24	2.99	3.68	2.66
Gäbriswald	AR	1175	218	M	753 430	249 915	24	2.48	3.63	2.97
Höchi	SG	970	302	M	725 865	234 945	24	2.66	4.12	2.51
Hasenried	SG	1171	381	M	740 520	233 400	24	2.53	3.78	2.55
Hausmösli 1	AR	940	539	L	740 935	242 245	24	2.92	3.41	2.40
Gäbrisseeli	AR	1020	979	L	753 945	249 895	48	2.60	3.61	2.63
Janseren	SZ	930	1422	M	691 700	214 785	48	2.56	3.48	2.56
Wäni	SZ	922	1604	M	699 245	218 905	48	2.94	3.91	2.49
Sulzel	SZ	956	1798	L	703 400	222 400	48	2.73	3.75	2.39
Steintal	SG	1040	2266	L	728 320	230 140	48	2.71	4.16	2.41
Grabserberg	SG	1335	2414	L	747 735	227 290	48	2.56	3.87	2.30
Chellen	SG	940	2740	L	730 475	237 395	48	2.74	4.12	2.13
Roblosen 1	SZ	897	3456	L	700 950	223 100	30	2.98	3.56	2.13
Hausmösli 2	AR	1200	3676	L	740 790	242 135	24	2.78	3.40	2.25
Etteren	SZ	1020	3683	M	697 020	213 180	48	2.85	3.68	2.78
Fetzeren	AR	1005	7671	L	735 900	241 015	60	2.73	3.39	2.52
Roblosen 2	SZ	900	8364	L	700 760	223 165	24	2.80	3.75	2.49
Wideralp (Schwägalp)	SG	1350	8479	L	741 465	234 620	48	2.52	3.94	2.53
Stein	SG	950	8562	M	733 655	228 725	48	2.68	3.58	2.60
Feusisberg	SZ	865	8824	M	697 570	224 390	48	2.97	3.69	2.86
Bannholz	SZ	947	10917	L	704 130	221 425	48	2.87	3.23	2.89
Landscheidi	AR	935	11325	M	734 638	240 576	30	2.83	3.19	2.74
Feldmoos	SG	890	14739	L	731 175	232 095	48	2.73	3.61	2.59
Munzenriet	SG	1030	43397	L	744 845	229 390	48	2.85	3.72	2.47

Demographic data and life cycle transitions

In May 2000, in 15 larger populations we established two blocks of 50 x 50 m at distances of 5 to 135 m from each other. We established only one such block in the 11 smaller populations. In each block, we marked 24-48 randomly selected *L. flos-cuculi* individuals with labels and established 18 x 18 cm plots around each of the target plants, which we marked with two 30 cm long iron nails stuck into the ground. We monitored the marked plants once a year from 2000 to 2004. We recognized each plant as vegetative, reproductive, or dead and used their diameter as life-cycle stage criteria.

For the matrix analysis we classified plants into six different life cycle stages. We considered them as seedling (S) when plants were still soft with a rosette diameter smaller than 4 cm. We defined four further stages of vegetative plants, juveniles (J) between ≥4 and <6 cm, Adult 1 (A1) between ≥6 and <9 cm, Adult 2 (A2) between ≥9 and <12 and Adult 3 (A3) larger than ≥12 cm. Finally, the reproductive stage (R) is represented by flowering plants.

Transition probabilities between these life stages were calculated following Caswell (2001) based on the life cycle diagram (Figure 1). Each transition rate was calculated by dividing the number of individual experiencing the given transition from *time t* to *time t+1 year* by the total of individuals counted in the considered stage at *time t*. All transitions, including retrogressions between vegetative states (between A3 and S), were observed at least once over all populations during the 5 years. Transitions including the seed bank were obtained from a sowing experiment in the field (Perret 2003a). This experiment had been conducted in 15 of our 26 populations. Here, for each of the 26 populations we used the mean germination values over these 15 populations. Germination rates had been measured 4 and 14 months after sowing. We calculated the transitions involving the seed bank as follows:

germ'01: germination rate after 4 months (mean value)

germ '02: germination rate after 14 months (mean value)

$$\mathbf{SB} \longrightarrow \mathbf{SB} = \sqrt{\frac{germ'02}{(1 - germ'01) * germ'01}}$$

$$\mathbf{R} \longrightarrow \mathbf{SB} = (1 - germ'01) * \sqrt{\frac{germ'02}{(1 - germ'01) * germ'01}}$$

$$\mathbf{SB} \longrightarrow \mathbf{S} = germ'01 * S -> S_{transition}$$

The transition rate from S to S was obtained from the matrix over all years and for all populations. Finally, the transition from R to S resulted from field counts of seedlings in the same 18 x 18 cm plots following a recorded reproductive plant the previous year. *L. flos-cuculi* plants disperse seeds by vibrations of the stiffened stalk, and consequently the majority of seeds stays in the direct vicinity of the maternal plant.

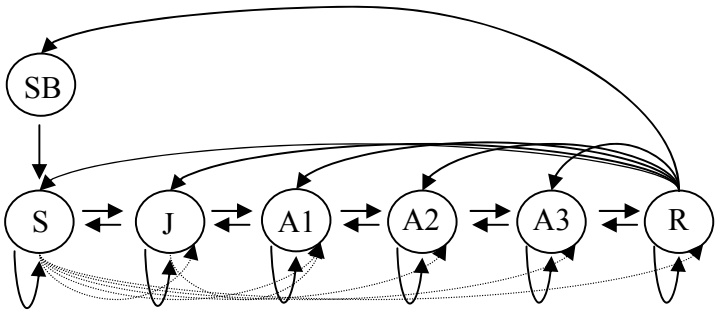

Figure 1: Life cycle diagram showing the stages used for the matrix model and the transitions observed during the study of 26 *Lychnis flos-cuculi* populations. To keep the diagram readable we only represented the more commonly observed transitions by arrows. However, over the 5 years of the study at least once we observed each transition between all the vegetative states (A3 to S), including retrogressions. Abbreviations: SB=Seed bank, S=Seedlings, A1=Adult 1, A2=Adult 2, A3=Adult 3, R=Reproductive.

Population growth rates

From our field data, we calculated all observable year-to-year transition probabilities (each element a_{ij} of the matrix represents the transition from the j^{th} category in *year t* to the i^{th} in *year t+1)* and organised them in Lefkovitch stage transition matrices.

We used the Lefkovitch matrix based on the 5 years of observations to calculate finite growth rates (λ) of populations with the mathematical program MATLAB (The language of technical computing, MathWorks, Inc., Natick, USA). Several matrices can be pooled by dividing the total number of plants experiencing a certain transition in any of the matrices to be pooled by the total number of plants in the initial stage of this transition in any matrix (Caswell 2001). We tested the relationship between the pooled lambdas (pooled for the whole time period, from 2000 to 2004) and the size and isolation of populations with ANOVA with S-PLUS (Version 6.1/6.2, 2002 Insightful Corp. Seattle, USA). Similarly, we tested the relationship of standard deviations and coefficients of variation of annual lambdas of each populations with the size and isolation of populations.

Moreover, with the software Karismat (K. Lehtilä, unpublished), we calculated stable stage vectors for four pooled matrices. Matrices of small populations were pooled by dividing the total number of plants of all small populations experiencing a certain transition in any year by the total number of plants in the initial stage of this transition in any year. Similarly, we pooled matrices for large, more isolated, and less isolated populations and we obtained stable stage vectors for these different types of populations.

Stochastic projection simulations

Finally, we carried out stochastic simulations with the software Karismat (K. Lehtilä, unpublished) using the 2001, 2002, 2003 and 2004 annual Lefkovitch matrices. We assumed that all annual matrices may apply with the same probability except for the transition between 2003 and 2004, which was very unusual because of the very dry summer of 2003. We assumed that such a summer only occurs every 25 years, and therefore applied the matrices with frequencies of 0.32, 0.32, 0.32, and 0.04 in our simulations, the latter for the unusual transition.

We did 1000 simulation runs of 50 years each to calculate finite population growth rates and extinction rates for all populations. From these data, we calculated average simulated rates of extinction and average simulated finite population growth rates for four different population types, small and less isolated, small and more isolated, large and less isolated, and large and more isolated. We tested the significance of effects of size and isolation of populations on simulated rates of extinction and average simulated finite population growth rates with ANOVA.

Results

Population characteristics

Population sizes, i.e. the numbers of flowering plants, of the 26 observed populations varied significantly between years (Table 2). In particular, population sizes were strongly reduced in 2003 (Figure 2). Populations with stronger reductions in population size between 2001 and 2003 experienced stronger increases between 2003 and 2004 (Figure 3).

Population growth rates

Finite growth rates of populations (λ) pooled over the 5 years varied between 1 and 1.04 with an average of 1.02 (Table 3; Appendix A). Differences in pooled λ between less and more isolated populations were not statistically significant. However, λ tended to be smaller for smaller populations (Table 4; Figure 4).

Table 2: ANOVA table of effects of population characteristics and environmental conditions on population sizes of the 26 study populations of *Lychnis flos-cuculi*. We present degrees of freedom (df), F, and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The correction factor ε after Huynh and Feldt was 0.9486.

Source of variation in Population sizes	**df**	**ms**	**F**	**p**
Altitude	1	3.3948	1.9165	0.1801
Light	1	0.3121	0.1762	0.6787
Moisture	1	2.6193	1.4787	0.2369
Nutrient	1	0.8485	0.4790	0.4961
Population	22	1.7713	8.8940	<0.001***
Year	3	8.6577	43.4712	<0.001***
Precipitation	1	0.0227	0.1140	0.7367
Residuals (=Population x Year)	60	0.1992		

Table 3: Finite population growth rates (λ) based on Lefkovitch matrices pooled over 5 years of monitoring, standard deviation and temporal coefficients of variation of annual λ, and finite population growth rate and extinction rate from stochastic simulations for the 26 *Lychnis flos-cuculi* populations of different size and isolation (M=more isolated, L=less isolated). S.D. and C.V. of annual λ were based on matrices of Appendix A, pooled λ were based on matrices of Appendix B.

	Population size	Isolation	Pooled λ	S.D of annual λ	C.V of annual λ	Stochastic λ	Extinction rate
Wäni	1604	M	1.0369	0.1844	0.1778	0.9551	1
Fetzeren	7671	L	1.0125	0.1821	0.1799	0.9132	0.963
Feusisberg	8824	M	1.0153	0.0724	0.0713	0.9116	0.073
Roblosen 1	3456	L	1.025	0.0670	0.0654	0.9782	0.99
Sulzel	1798	L	1.0268	0.0748	0.0729	0.9665	0
Bannholz	10917	L	1.0449	0.1014	0.0971	0.9551	0
Janseren	1422	M	1.0002	0.0468	0.0468	0.9207	1
Höchi	302	M	1	0.0642	0.0642	0.9625	0
Allmeindswald	204	M	1.0167	0.0774	0.0761	0.9098	0.048
Chellen	2740	L	1.0146	0.0908	0.0895	0.9206	1
Hausmösli 1	539	L	1.0102	0.0670	0.0663	0.9846	0.999
Hausmösli 2	3676	L	1.0072	0.0670	0.0665	0.9207	0.392
Gäbriswald	218	M	1.0217	0.0774	0.0757	0.9127	0.056
Gäbrisseeli	979	L	1.016	0.0582	0.0573	0.9137	0.004
Etteren	3683	M	1.0246	0.0670	0.0654	0.9846	0
Steintal	2266	L	1.0134	0.0670	0.0661	0.9531	0.003
Feldmoos	14739	L	1.0055	0.0102	0.0101	0.9503	0
Stein	8562	M	1.0338	0.0856	0.0828	0.9079	0.12
Munzenriet	43397	L	1.0237	0.0647	0.0632	0.9098	0.995
Hasenried	381	M	1.0079	0.0647	0.0641	0.9650	0.002
Wideralp (Schwägalp)	8479	L	1.0101	0.0009	0.0009	0.9098	1
Bühler	119	M	1.0015	0.0774	0.0772	0.9200	0.214
Grabserberg	2414	L	1.0228	0.0914	0.0894	0.9098	1
Seilerzwecken	116	M	1.0093	0.0670	0.0664	0.9313	0.008
Roblosen 2	8364	L	1.0136	0.0670	0.0661	0.9551	1

Table 4: Summary of analyses of variance of finite growth rates (λ) based on Lefkovitch matrices pooled over 5 years of monitoring, standard deviation and temporal coefficient of variation of annual λ, and finite population growth rate and extinction rate from stochastic simulations for the 26 *Lychnis flos-cuculi* populations of different size and isolation (M=more isolated, L=less isolated). We present degrees of freedom (df), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

	λ simulated				**Extinction rates**			
Source of variation	df	ms	F	p	df	ms	F	p
Population size	1	0.0016	2.0773	0.1650	1	10795	8.1547	0.0098**
Isolation	1	0.0001	0.0998	0.7553	1	14.61	0.0110	0.9174
Population size x Isolation	1	0.0003	0.3643	0.5529	1	2097.7	1.5846	0.2226
Residuals (= Population)	20	0.0007			20	1323.8		

	λ pooled				**Standard deviation λ 2000-2004**				**Coefficient of variation of λ 2000-2004**			
Source of variation	df	ms	F	p	df	ms	F	p	df	ms	F	p
Population size	1	0.0004	3.4228	0.0784^{+}	1	0.0003	0.2004	0.6590	1	0.0004	0.2427	0.6274
Isolation	1	0.0001	0.8179	0.3760	1	0.0001	0.0813	0.7783	1	0.0001	0.0658	0.8001
Population size x Isolation	1	0.0000	0.2192	0.6445	1	0.0016	0.9967	0.3295	1	0.0016	1.0145	0.3253
Residuals (= Population)	21	0.0001			21	0.0016			21	0.0015		

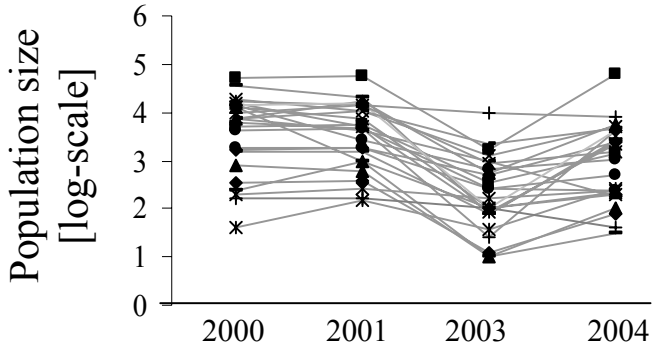

Figure 2: Population sizes of *Lychnis flos-cuculi* varied significantly between the years 2000 and 2004 (Table 2). In particular, we observed a strong decrease of population sizes in the dry year 2003.

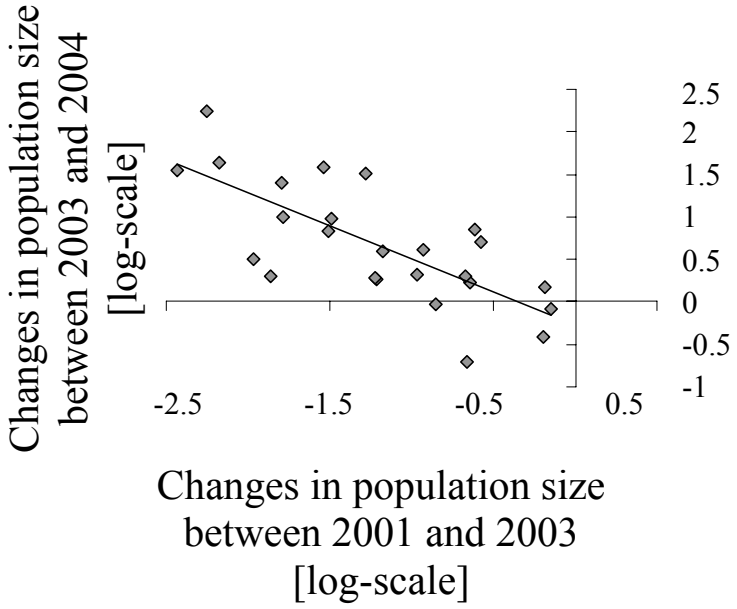

Figure 3: Populations with the strongest reduction in number of individuals between 2001 and 2003 showed the strongest increase in number of individuals between 2003 and 2004 ($r = -0.73$, $p<0.0001$).

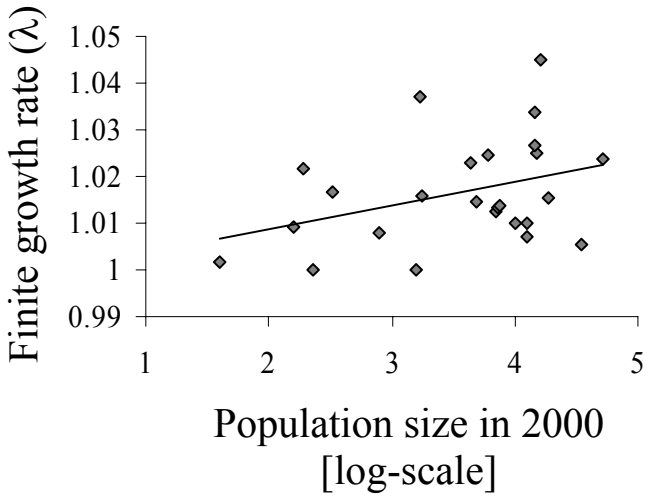

Figure 4: Mean annual finite population growth rates (λ) of 24 of the 26 study populations of *Lychnis flos-cuculi* varied between 1 and 1.04 and tended to be smaller for populations with smaller initial population sizes ($r = 0.367$, $p = 0.07$).

Proportions of plants in different stages

Differences between observed and projected stable vectors were highly significant due to an excess of larger and older plants (X^2-test; $p < 0.001$) for all populations. This also held true when we analysed the four groups of populations separately, i.e. larger (X^2-test; $p<0.001$), smaller (X^2-test; $p<0.001$), less isolated (X^2-test; $p<0.001$), and more isolated (X^2-test; $p<0.001$) populations (Appendix B; Figure 5).

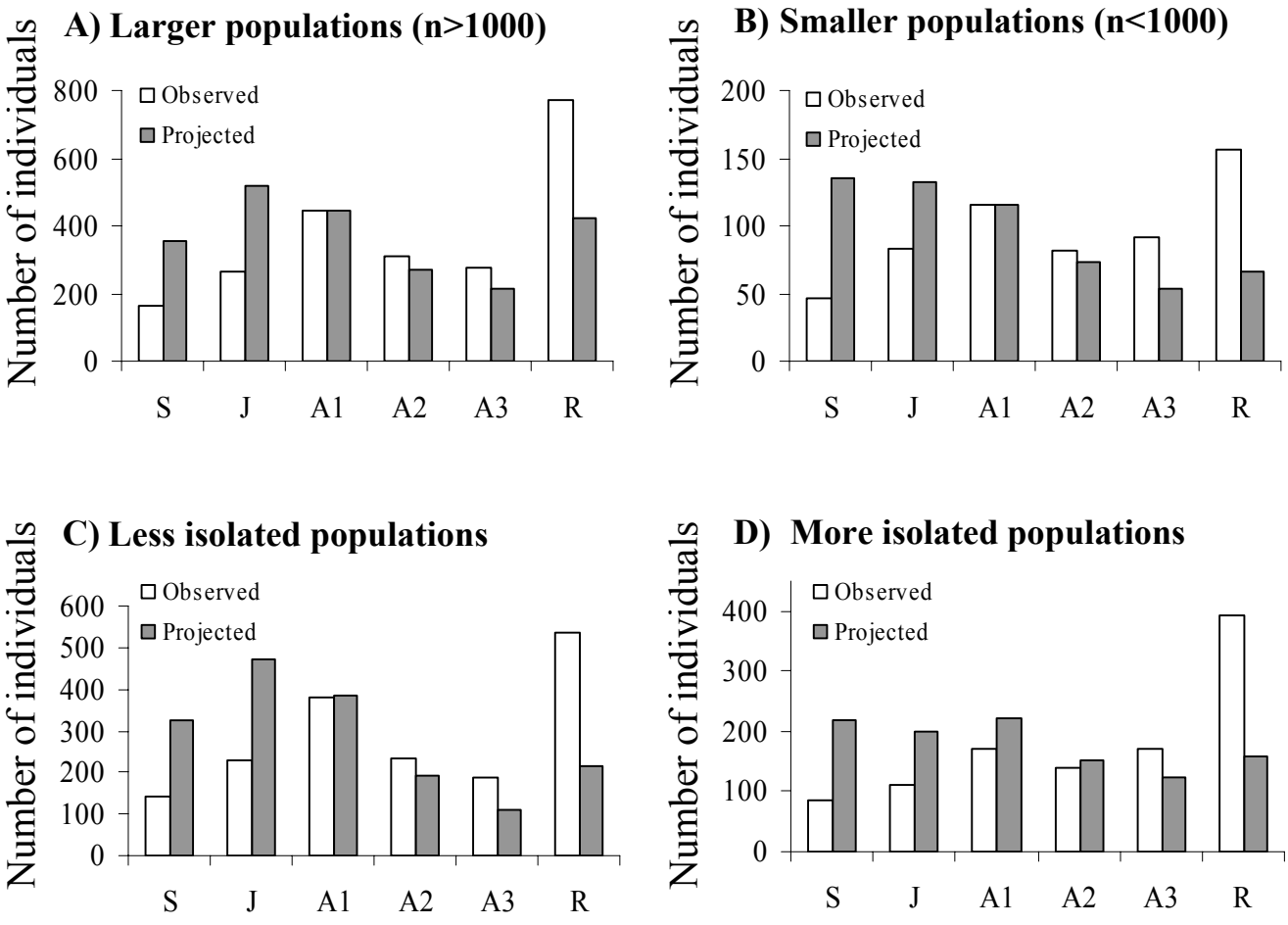

Figure 5: Observed and projected stable stage vectors for the 26 study populations of *Lychnis flos-cuculi* grouped into A) 16 larger populations, B) 8 smaller populations, C) 13 less isolated populations, and D) 11 more isolated populations. Differences between observed and projected vectors were highly significant for each group (X^2-test; $p < 0.001$), due to an excess of larger and older plants.

Simulation models

The simulated number of individuals rapidly decreased in all types of populations (see Figure 6 for an illustration). Mean finite population growth rates calculated over 1000 simulation runs were lower than 1 for all types of population (Figure 7A). Moreover, mean extinction rates over 1000 simulations were significantly higher for smaller populations (Table 4; Figure 7B, C).

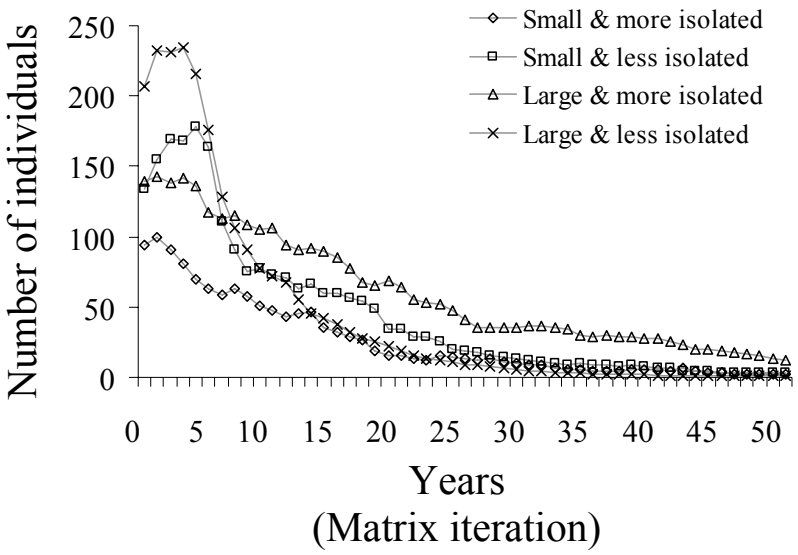

Figure 6: Illustration of the stochastic simulations of population growth for 50 years. The graph shows one simulation run for one example population of the four types of populations of *Lychnis flos-cuculi.* These example populations were the ones with median extinction rate in Figure 7B.

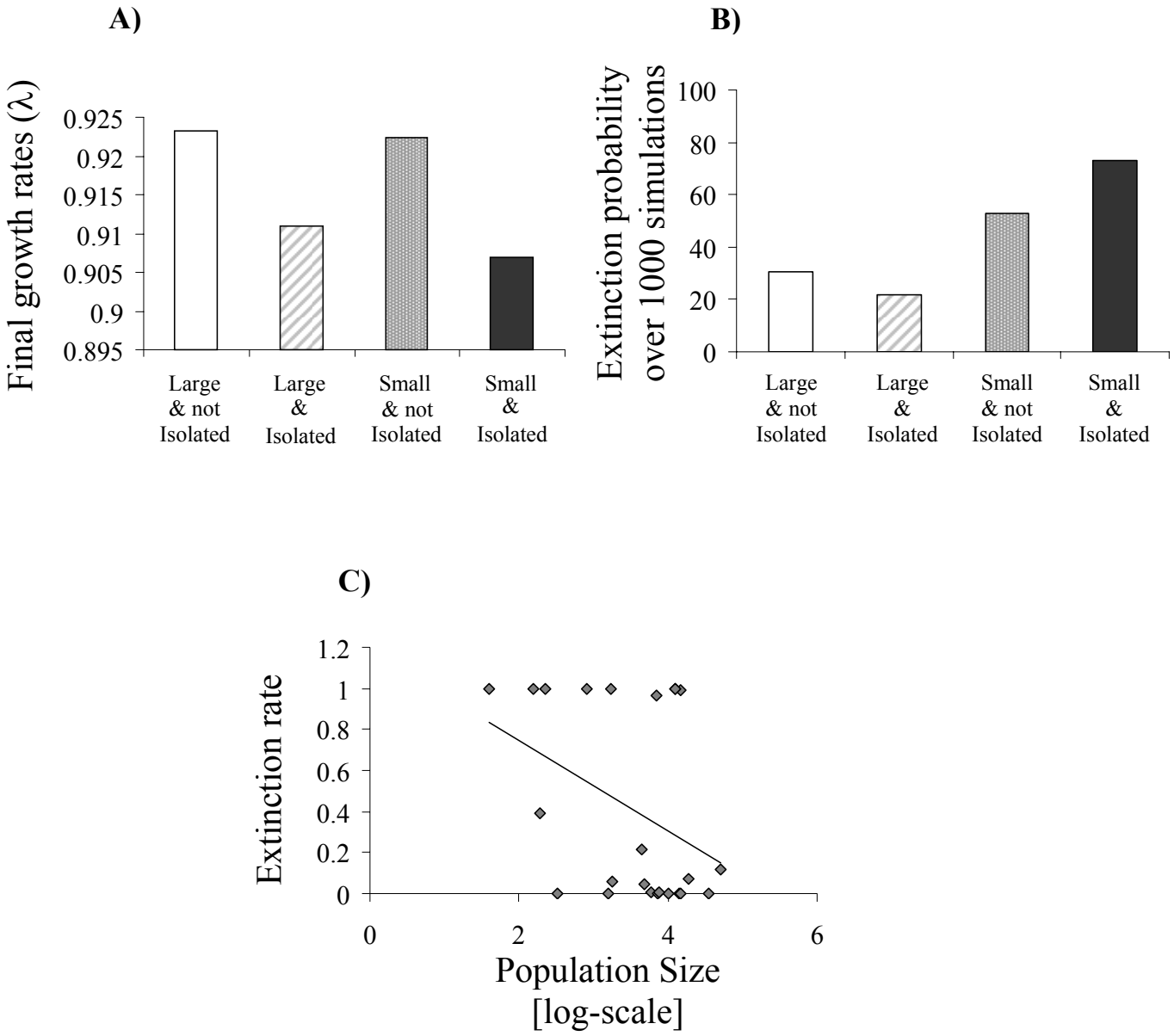

Figure 7: Finite growth rates A) and extinction rates B) of populations predicted from simulations based on field-data based transition matrices pooled for four different groups of populations according to population size and isolation. C) Simulated extinction rates as function of initial population size. Graphs are based on mean values of 1000 simulations of 50 years each.

Discussion

Population sizes and environmental conditions

The numbers of flowering plants per population differed significantly between years and between populations (Table 2). A third of the populations were consistently smaller than 1000 flowering individuals, i.e. smaller than the frequently suggested threshold size below which Allee effects become particularly pronounced (Fischer 1996). The observed significant decrease of the numbers of flowering plants per population in the dry year 2003 (Figure 2) and the higher increase in these numbers in 2004 for populations which had experienced the strongest decrease (Figure 3), suggest a compensatory response of populations. In accordance with results of Sandvik for *Saxifraga stellaris* (Sandvik 2001), we hypothesize that this was due to the ability of those plants, which refrained from reproduction in 2003 in response to drought, to especially increase their growth and reproduction in the following year (Sandvik 2001).

However, by the end of our field study in 2004 population sizes had not reached the levels observed in 2001, suggesting that especially the dry summer of 2003 had generated higher mortality in plants. This overall decrease in population size did not significantly differ between large and small populations or between isolated and less isolated populations. It may well be that the longer term will reveal that the dry year 2003 exemplifies stochastic events reducing population sizes to a level where their future development is threatened by Allee effects.

Mean population growth rates and temporal variation

The monitoring of individual plants analyzed with matrix modeling revealed finite rates of population growth (λ) very close to 1 for all populations, suggesting relatively stable populations. However, because population growth rates are generally not constant between years, and also varied between the years of our study for all populations (Table 3), populations may decline over years even if the predicted mean λ is 1 or slightly higher than 1.

Stage structures of populations

Stable stage structure of perennial plants are commonly characterized by a high proportion of smaller size classes compared to bigger ones (Mondragón et al. 2004). We observed significant differences between observed and projected stable stage vectors (Figure 5), where individuals in younger stages of the life cycle were less frequent than predicted. This suggests that the stable stage distribution recently was disturbed by some environmental stochastic events or that vital rates have changed, for example due to climate modifications, and that the new stable stage structure has not yet been reached (Barot et al. 2000). The deviation of the demographic structure observed in our *Lychnis flos-cuculi* populations suggests that these populations are in a regressive state (Oostermeijer et al. 1992).

Predicted extinction rates

Smaller populations of *L. flos-cuculi* tended to have lower average λ than larger populations, supporting earlier findings of Fischer and Matthies (1998) for the biennial *Gentianella germancia* in landscapes with fragmented nutrient-poor calcareous grasslands. A smaller mean population growth rate of smaller populations (Figure 4) combined with the finding of similar temporal variation in population growth rates between small and large populations (Table 3; Table 4), suggest that in the longer run smaller populations face higher risks of local extinction than larger ones. This was indeed confirmed in our stochastic simulations, which predict significantly higher extinction rates for initially smaller populations (Table 3; Table 4; Figure 7).

CONCLUSION

Overall, our results clearly indicate, that if environmental and demographic situation of populations do not change, populations of *L. flos-cuculi* will continue their decline. Especially small populations, which actually constitute a considerable proportion of all populations, are at high risks of local extinction. We conclude that predictions based on short-term data, according to which natural populations of common plants are also affected by landscape fragmentation, are supported by our longer-term demographic study.

Acknowledgments

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Appendix A

Population transition matrices for all sites for the period 2000-2004. The population growth rates λ are given above each matrix.

Population Wäni 2000-2004

λ = 1.037

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.000	0.000	0.056	0.038
J	0.000	0.000	0.000	0.111	0.222	0.000	0.151
A1	0.000	0.000	0.167	0.444	0.333	0.056	0.132
A2	0.000	1.000	0.500	0.000	0.111	0.056	0.132
A3	0.000	0.000	0.333	0.222	0.111	0.278	0.151
R	0.000	0.000	0.000	0.222	0.222	0.556	0.396

Population Gäbriswald 2000-2004

λ = 1.022

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.500	0.000	0.000	0.115
J	0.000	0.000	0.333	0.000	0.000	0.100	0.385
A1	0.000	0.667	0.667	0.000	0.167	0.200	0.192
A2	0.000	0.000	0.000	0.125	0.333	0.100	0.038
A3	0.000	0.333	0.000	0.375	0.167	0.100	0.077
R	0.000	0.000	0.000	0.000	0.333	0.500	0.192

Population Höchi 2000-2004

λ = 1

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	1.000	0.250	0.000	0.000	0.000	0.083
J	0.000	0.000	0.438	0.400	0.000	0.333	0.292
A1	0.000	0.000	0.125	0.200	0.000	0.000	0.167
A2	0.000	0.000	0.000	0.000	0.500	0.000	0.125
A3	0.000	0.000	0.063	0.200	0.500	0.333	0.042
R	0.000	0.000	0.125	0.200	0.000	0.333	0.292

Population Landscheidi 2000-2002

λ = 1

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	1.000	0.000	0.500	0.000	0.000	0.313
J	0.000	0.000	0.556	0.167	0.000	1.000	0.438
A1	0.000	0.000	0.333	0.000	0.500	0.000	0.000
A2	0.000	0.000	0.111	0.000	0.000	0.000	0.125
A3	0.000	0.000	0.000	0.000	0.000	0.000	0.000
R	0.000	0.000	0.000	0.333	0.500	0.000	0.125

Population Gäbrisseeli 2000-2004

λ = 1.016

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.200	0.286	0.056	0.000	0.000	0.273
J	0.000	0.400	0.357	0.306	0.000	0.462	0.364
A1	0.000	0.000	0.214	0.306	0.400	0.154	0.182
A2	0.000	0.200	0.000	0.167	0.200	0.000	0.136
A3	0.000	0.200	0.071	0.083	0.000	0.000	0.045
R	0.000	0.000	0.071	0.083	0.400	0.385	0.000

Population Allmeindswald 2000-2004

λ = 1.017

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.000	0.000	0.250	0.167
J	0.000	0.333	0.250	0.286	0.000	0.000	0.167
A1	0.000	0.333	0.250	0.286	0.167	0.125	0.125
A2	0.000	0.333	0.250	0.429	0.417	0.250	0.083
A3	0.000	0.000	0.250	0.000	0.167	0.375	0.167
R	0.000	0.000	0.000	0.000	0.250	0.000	0.292

Population Fetzeren 2000-2004

λ = 1.012

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.688	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.400	0.300	0.120	0.074	0.000	0.225
J	0.000	0.200	0.400	0.160	0.296	0.000	0.313
A1	0.000	0.400	0.100	0.320	0.037	0.375	0.125
A2	0.000	0.000	0.000	0.080	0.037	0.125	0.113
A3	0.000	0.000	0.000	0.000	0.148	0.000	0.025
R	0.000	0.000	0.200	0.320	0.407	0.500	0.200

Population Etteren 2000-2004

λ = 1.025

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.500	0.000	0.150	0.059	0.000	0.216
J	0.000	0.000	0.000	0.200	0.000	0.042	0.275
A1	0.000	0.000	0.333	0.450	0.235	0.083	0.157
A2	0.000	0.000	0.333	0.150	0.118	0.167	0.039
A3	0.000	0.500	0.333	0.050	0.353	0.208	0.118
R	0.000	0.000	0.000	0.000	0.235	0.500	0.196

Population Chellen 2000-2004

λ = 1.015

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.444	0.154	0.167	0.000	0.300
J	0.000	0.667	0.222	0.154	0.167	0.000	0.175
A1	0.000	0.333	0.111	0.308	0.083	0.200	0.100
A2	0.000	0.000	0.222	0.077	0.167	0.200	0.125
A3	0.000	0.000	0.000	0.154	0.250	0.300	0.125
R	0.000	0.000	0.000	0.154	0.167	0.300	0.175

Population Feusisiberg 2000-2004

λ = 1.015

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.400	0.100	0.167	0.286	0.000	0.133
J	0.000	0.200	0.600	0.167	0.000	0.000	0.567
A1	0.000	0.400	0.200	0.333	0.000	0.000	0.133
A2	0.000	0.000	0.100	0.167	0.000	0.000	0.100
A3	0.000	0.000	0.000	0.167	0.000	0.000	0.000
R	0.000	0.000	0.000	0.000	0.714	1.000	0.067

Population Steintal 2000-2004

λ = 1.013

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.125	0.167	0.067	0.000	0.000	0.182
J	0.000	0.500	0.333	0.200	0.571	0.000	0.409
A1	0.000	0.125	0.167	0.533	0.286	0.133	0.136
A2	0.000	0.000	0.333	0.067	0.000	0.133	0.045
A3	0.000	0.125	0.000	0.000	0.143	0.200	0.045
R	0.000	0.125	0.000	0.133	0.000	0.533	0.182

Population Hausmösli 1 2000-2004

λ = 1.01

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.500	0.167	0.000	0.000	0.000	0.286
J	0.000	0.500	0.333	0.143	0.000	1.000	0.214
A1	0.000	0.000	0.333	0.714	0.500	0.000	0.214
A2	0.000	0.000	0.167	0.143	0.000	0.000	0.071
A3	0.000	0.000	0.000	0.000	0.000	0.000	0.000
R	0.000	0.000	0.000	0.000	0.500	0.000	0.214

Population Roblosen 1 2000-2004

λ = 1.025

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.250	0.000	0.200	0.091	0.000	0.136
J	0.000	0.000	0.000	0.000	0.091	0.000	0.273
A1	0.000	0.500	0.000	0.200	0.182	0.500	0.091
A2	0.000	0.000	1.000	0.200	0.000	0.000	0.182
A3	0.000	0.000	0.000	0.400	0.182	0.250	0.136
R	0.000	0.250	0.000	0.000	0.455	0.250	0.182

Population Feldmoos 2000-2004

λ = 1.001

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.769	0.238	0.250	0.200	0.000	0.173
J	0.000	0.231	0.190	0.375	0.267	1.000	0.423
A1	0.000	0.000	0.381	0.225	0.267	0.000	0.192
A2	0.000	0.000	0.143	0.050	0.133	0.000	0.077
A3	0.000	0.000	0.000	0.025	0.000	0.000	0.000
R	0.000	0.000	0.048	0.075	0.133	0.000	0.135

Population Hausmösli 2 2000-2004

λ = 1.007

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.200	0.286	0.000	0.333	0.000	0.259
J	0.000	0.600	0.714	0.143	0.000	0.000	0.407
A1	0.000	0.000	0.000	0.714	0.000	0.500	0.148
A2	0.000	0.200	0.000	0.000	0.000	0.000	0.000
A3	0.000	0.000	0.000	0.000	0.000	0.000	0.000
R	0.000	0.000	0.000	0.143	0.667	0.500	0.185

Population Sulzel 2000-2004

λ = 1.027

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.036	0.000	0.000	0.138
J	0.000	0.200	0.500	0.429	0.154	0.000	0.154
A1	0.000	0.200	0.167	0.357	0.308	0.059	0.185
A2	0.000	0.200	0.167	0.143	0.000	0.000	0.108
A3	0.000	0.000	0.167	0.036	0.231	0.412	0.154
R	0.000	0.400	0.000	0.000	0.308	0.529	0.262

Population Stein 2000-2004

λ = 1.034

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.286	0.000	0.125	0.079
J	0.000	0.500	0.222	0.143	0.000	0.125	0.238
A1	0.000	0.000	0.444	0.286	0.111	0.000	0.111
A2	0.000	0.500	0.111	0.143	0.222	0.125	0.143
A3	0.000	0.000	0.111	0.000	0.111	0.125	0.111
R	0.000	0.000	0.111	0.143	0.556	0.500	0.317

Population Wideralp (Schwägalp) 2000-2004

λ = 1.01

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.182	0.348	0.107	0.083	0.118	0.277
J	0.000	0.364	0.217	0.286	0.000	0.176	0.538
A1	0.000	0.273	0.261	0.179	0.333	0.176	0.123
A2	0.000	0.091	0.174	0.214	0.083	0.176	0.046
A3	0.000	0.091	0.000	0.179	0.333	0.059	0.000
R	0.000	0.000	0.000	0.036	0.167	0.294	0.015

Population Bannholz 2000-2003

λ = 1.045

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.000	0.000	0.348	0.357
J	0.000	0.000	0.000	0.000	0.000	0.391	0.089
A1	0.000	0.000	1.000	0.000	0.500	0.130	0.089
A2	0.000	0.000	0.000	0.250	0.000	0.000	0.036
A3	0.000	0.333	0.000	0.000	0.500	0.000	0.125
R	0.000	0.667	0.000	0.750	0.000	0.130	0.304

Population Janseren 2000-2003

λ = 1

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.333	0.222	0.067	0.100	0.150
A1	0.000	0.000	0.500	0.222	0.267	0.200	0.350
A2	0.000	0.000	0.167	0.278	0.333	0.300	0.125
A3	0.000	0.000	0.000	0.000	0.067	0.100	0.025
R	0.000	0.000	0.000	0.278	0.267	0.300	0.350

Population Roblosen 2 2000-2004

λ = 1.014

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.400	0.333	0.000	0.000	0.000	0.205
J	0.000	0.000	0.500	0.176	0.400	0.000	0.333
A1	0.000	0.600	0.000	0.529	0.100	0.500	0.282
A2	0.000	0.000	0.167	0.059	0.300	0.000	0.026
A3	0.000	0.000	0.000	0.118	0.000	0.000	0.000
R	0.000	0.000	0.000	0.118	0.200	0.500	0.154

Population Munzenriet 2000-2004

λ = 1.024

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.231	0.143	0.000	0.000	0.091
J	0.000	0.400	0.154	0.321	0.375	0.000	0.491
A1	0.000	0.200	0.308	0.143	0.125	0.231	0.164
A2	0.000	0.200	0.154	0.143	0.188	0.077	0.145
A3	0.000	0.000	0.077	0.107	0.031	0.000	0.000
R	0.000	0.200	0.077	0.143	0.281	0.692	0.109

Population Hasenried 2000-2004

λ = 1.008

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.636	0.200	0.222	0.250	0.400	0.348
J	0.000	0.182	0.400	0.333	0.250	0.200	0.435
A1	0.000	0.091	0.300	0.222	0.250	0.200	0.000
A2	0.000	0.000	0.000	0.000	0.000	0.000	0.043
A3	0.000	0.091	0.000	0.222	0.000	0.000	0.000
R	0.000	0.000	0.100	0.000	0.250	0.200	0.174

Population Seilerzwecken 2000-2004

λ = 1.009

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.800	0.000	0.000	0.400	0.600	0.065
J	0.000	0.000	0.000	0.000	0.000	0.000	0.258
A1	0.000	0.000	0.000	0.000	0.000	0.100	0.097
A2	0.000	0.200	0.000	1.000	0.200	0.000	0.065
A3	0.000	0.000	0.000	0.000	0.400	0.100	0.032
R	0.000	0.000	1.000	0.000	0.000	0.200	0.484

Population Bühler 2000-2004

λ = 1.001

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.000	0.000	0.200	0.000	0.000	0.400
J	0.000	0.000	0.000	0.000	0.000	0.333	0.133
A1	0.000	0.000	0.000	0.800	0.000	0.167	0.100
A2	0.000	1.000	0.000	0.000	0.500	0.167	0.100
A3	0.000	0.000	0.000	0.000	0.000	0.000	0.067
R	0.000	0.000	0.000	0.000	0.500	0.333	0.200

Population Grabserberg 2000-2004

λ = 1.022

temps t+1 \ *temps t*	SB	S	J	A1	A2	A3	R
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683
S	0.032	0.167	0.143	0.182	0.048	0.000	0.169
J	0.000	0.167	0.000	0.182	0.048	0.000	0.308
A1	0.000	0.333	0.286	0.273	0.143	0.083	0.215
A2	0.000	0.167	0.143	0.227	0.333	0.333	0.108
A3	0.000	0.000	0.286	0.091	0.190	0.333	0.031
R	0.000	0.167	0.143	0.045	0.238	0.250	0.169

Appendix B

Pooled transition matrices and stable stage distribution vector for the period 2000-2004. Population matrices were grouped for small (n<1000), large (n>1000), less isolated and more isolated populations.

Less isolated populations 2000-2004

temps t+1	SB	S	J	A1	A2	A3	R	Stable stage vector
SB	0.688	0.000	0.000	0.000	0.000	0.000	0.683	0.151
S	0.032	0.275	0.264	0.112	0.063	0.016	0.200	0.218
J	0.000	0.325	0.287	0.266	0.217	0.131	0.347	0.179
A1	0.000	0.213	0.225	0.324	0.177	0.180	0.164	0.090
A2	0.000	0.075	0.140	0.122	0.137	0.107	0.092	0.051
A3	0.000	0.038	0.039	0.076	0.126	0.156	0.042	0.100
R	0.000	0.075	0.047	0.101	0.280	0.410	0.154	0.787

More isolated populations 2000-2004

temps t+1	SB	S	J	A1	A2	A3	R	Stable stage vector
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683	0.123
S	0.032	0.410	0.118	0.156	0.080	0.131	0.154	0.112
J	0.000	0.154	0.338	0.178	0.040	0.112	0.271	0.125
A1	0.000	0.154	0.265	0.367	0.173	0.093	0.127	0.087
A2	0.000	0.205	0.118	0.133	0.200	0.112	0.092	0.070
A3	0.000	0.077	0.088	0.122	0.187	0.187	0.084	0.089
R	0.000	0.000	0.074	0.044	0.320	0.364	0.271	0.606

Large populations 2000-2004

temps t+1	SB	S	J	A1	A2	A3	R	Stable stage vector
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683	0.104
S	0.032	0.271	0.210	0.120	0.064	0.048	0.164	0.153
J	0.000	0.294	0.280	0.246	0.186	0.151	0.321	0.131
A1	0.000	0.224	0.245	0.322	0.173	0.133	0.164	0.079
A2	0.000	0.106	0.168	0.126	0.145	0.187	0.096	0.062
A3	0.000	0.035	0.056	0.076	0.141	0.458	0.058	0.124
R	0.000	0.071	0.042	0.110	0.291	1.000	0.196	0.653

Small populations 2000-2004

temps t+1	SB	S	J	A1	A2	A3	R	Stable stage vector
SB	0.866	0.000	0.000	0.000	0.000	0.000	0.683	0.155
S	0.032	0.438	0.204	0.115	0.070	0.176	0.211	0.152
J	0.000	0.188	0.370	0.244	0.023	0.235	0.278	0.131
A1	0.000	0.125	0.241	0.321	0.209	0.132	0.129	0.083
A2	0.000	0.156	0.037	0.154	0.279	0.088	0.082	0.061
A3	0.000	0.094	0.056	0.115	0.140	0.132	0.057	0.075
R	0.000	0.000	0.093	0.051	0.279	0.235	0.242	0.658

Chapter 4

Fitness Consequences of Three Generations of Experimental Inbreeding of *Lychnis flos-cuculi* Plants from 19 Populations of a Fragmented Landscape

With Gillianne Bowman,

Catherine Perret and Markus Fischer

Abstract

In the fragmented landscape, isolation of populations and small population size increase rates of inbreeding within populations. This may lower plant fitness via inbreeding depression. However, depending on population history inbreeding load may differ between populations. It can change over generations because of dominance and epistasis or because of selection against deleterious alleles. We tested the consequences of three generations of crosses of different degrees of experimental selfing and outcrossing for plants of the self-compatible perennial plant species *Lychnis flos-cuculi* from 19 populations in North-East Switzerland. These populations of plant origin differed in size and genetic diversity. Inbreeding depression was expressed in all generations and at the stages of seed production, germination and adult plant size. Genetic load was substantial in small populations and it was not purged rapidly under selfing. While few interactions between levels of experimental inbreeding and genetic variation of the population of origin suggested purging of inbreeding load from genetically less variable natural populations, others did not. We conclude that common plants, such as *Lychnis flos-cuculi,* do not rapidly purge their inbreeding load if populations get small and are therefore prone to negative genetics consequences of landscape fragmentation.

Introduction

Nowadays, habitat fragmentation threatens the survival of many species because their populations become small and more isolated (Young et al. 1996, Stephens and Sutherland 1999, Serrano et al. 2002, Hooftman and Diemer 2003, Hooftman et al. 2004, Lienert 2004). For natural populations a reduction in size is associated with several demographic and genetic consequences including an increase of random genetic drift and an increase in inbreeding rate (Oostermeijer et al. 1994, Oostermeijer 1996, Young et al. 1996).

Allee effects are defined as mechanisms which lead to a positive relationship between individual fitness traits and the number or density of conspecifics in a population (Young et al. 1996). Some Allee effects are genetically based. One mechanism that can lead to genetic Allee effects, in combination with increased rates of inbreeding in small populations, is inbreeding depression. Inbreeding depression occurs when plant fitness is reduced by the breakdown of heterozygote advantages (overdominance in one or several loci) or by the expression of recessive deleterious alleles (Dudash 1990). The strength of inbreeding depression can differ between different stages of the plant life cycle. Expression of lethal or severely deleterious recessive alleles is supposed to happen in the earliest stages of the life cycle resulting in seed abortion or seedling mortality, while less deleterious alleles might be accumulated and decrease plant fitness later in the life cycle by reducing growth rate or reproduction (Lande and Schemske 1985, Dudash 1990, Hauser and Loeschcke 1994, Cheptou et al. 2000)

However, inbreeding depression does not necessary lead to Allee effects. In the long run, inbreeding load may be purged by selection against plants homozygous for detrimental alleles (Dole and Ritlant 1993, Hauser and Loeschcke 1994, Young et al. 1996, Carr and Dudash 1997). In smaller populations with lower genetic variability and higher inbreeding rates (Hauser and Loeschcke 1994, Oostermeijer 1996, Byers and Waller 1999, Kephart et al. 1999, Gibbs 2001, Hooftman et al. 2004) inbreeding load might be purged more rapidly by selection than in larger populations.

Genetic Allee effects need not only be caused by inbreeding and inbreeding depression, but also by the fixation of deleterious alleles which is more likely in smaller

populations. Once deleterious alleles are fixed in a population, they cannot be purged anymore by selection.

Our model plant species *Lychnis flos-cuculi* (L.) (Caryophyllaceae) is a common perennial plant of sunny and moist-to-wet meadows. In Switzerland, wetlands were reduced by 90% during the last century (Broggi and Schlegel 1989). Thus, *Lychnis flos-cuculi* has suffered from habitat destruction and fragmentation for several decades. A microsatellite study revealed that levels of within population inbreeding, F_{is}, are high (Galeuchet et al. 2005a) and genetic variation is reduced in smaller populations of *Lychnis flos-cuculi*. Moreover, plants from smaller populations perform worse in the greenhouse, indicating genetic Allee effects (Galeuchet et al. 2005b).

We focus on effects of inbreeding on the fitness of *L. flos-cuculi* plants from small and large populations. More specifically, we address the following questions: 1-How is *L. flos-cuculi* affected by inbreeding at different stages of its life cycle? 2-What are the consequences of longer-term inbreeding for *L. flos-cuculi?* 3-Are plant populations able to purge their genetic load, and if so, do smaller and more inbred populations purge their genetic load more efficiently than larger, more genetically variable populations ?

Material & Methods

Study Species

Lychnis flos cuculi L. (=*Silene flos cuculi* (L.) Clairv. Caryophyllaceae) is a common polycarpic perennial herb of sunny and moist-to-wet meadows. It is widespread from the plains to montane elevations across most of Switzerland and Europe (Aeschimann and Burdet 1994, Delarze et al. 1998, Lauber and Wagner 2000). Traditionally the species occurred abundantly in wet hay meadows or calcareous fens but in recent decades these habitats were severely fragmented (Broggi and Schlegel 1989, Keller 1996). Therefore, the species has recently declined in number and size of populations.

During the first growing season, a primary rosette is formed and usually several secondary rosettes grow from axillary buds. During the second growing season, one or more rosettes can become reproductive and grow flowering stems of 30-90 cm height (Lauber and Wagner 2000). The inflorescence is a dichasium with about 20 flowers.

Flowering season in the study area is in June and July. Flowers are typically protandrous, favouring outbreeding, but geitonogamous, self-pollinations do occur. Main pollinators are bumblebees (*Bombus* sp.) and Diptera, occasionally also Lepidoptera (Noctuidea) (Vejsnæs and Høvsgaad 1990, Hauser and Loeschcke 1994).

Fruits mature within 3 to 6 weeks (Galeuchet 2003). Mature fruit capsules, which contain up to 200 seeds, open at the top and seeds are dispersed when stalks are shaken by wind or animals.

Study Populations

For this study, we selected plants of 19 wild populations. All populations were located at altitudes between 860 m and 1200 m in the North-East of Switzerland, in the Cantons St-Gallen, Schwyz, and Appenzell (Table 1). The populations were situated in fen meadows mown yearly in September and not supplied with fertilizers. Population sizes were estimated by counting the number of flowering individuals at peak of flowering during June and July of 2000, 2001, 2003 and 2004. In 2004 estimated population sizes ranged from 30 to 63'000 flowering stems.

Microsatellite variation

For each population, estimates of Allelic richness, AR, and inbreeding coefficient, F_{is}, were available from a study of 18 plants per population with seven polymorphic microsatellite loci (Galeuchet *et al*., 2005). Within-population inbreeding coefficient F_{is} varied from 0.316 to 0.585 with an average of 0.467, indicating a mixed mating system with substantial inbreeding in *L. flos-cuculi*. Allelic richness, A_R, varied between 4.4 and 5.2 with an average of 4.8 over all populations (Table 1) and increased with increasing population size.

Table 1: Canton (AR = Appenzell Ausserrohden, SG = St-Gallen, SZ = Schwyz), Population size (average size from 2000 to 2004), altitude, Swiss geographic coordinates of the 19 *Lychnis flos-cuculi* populations studied and respective microsatellite allelic richness (AR) and inbreeding coefficient (F_{is}) (Galeuchet et al. 2005a).

Site	Canton	Altitude (m)	Coordinates		Population size	Sample size	Allelic richness AR	Mean inbreeding coefficient F_{is}
			E	N				
Allmeindswald	SG	1080	732 220	237 525	204	15.571	5.0	0.335
Gäbriswald	AR	1175	753 430	249 915	218	15.714	4.8	0.525
Höchi	SG	970	725 865	234 945	302	16.714	4.4	0.448
Hasenried	SG	1185	740 520	233 400	381	16.714	4.7	0.529
Haumösli 1	AR	940	740 935	242 245	539	8.286	4.7	0.452
Gäbrisseeli	AR	1020	753 945	249 895	979	13.000	4.5	0.510
Janseren	SZ	930	691 700	214 785	1422	16.143	4.4	0.416
Wäni	SZ	922	699 245	218 905	1604	15.571	4.9	0.467
Sulzel	SZ	956	703 400	222 400	1798	16.429	4.6	0.472
Chellen	SG	940	730 475	237 395	2740	17.143	5.2	0.427
Roblosen 1	SZ	897	700 950	223 100	3456	8.286	4.7	0.469
Etteren	SZ	1050	697 020	213 180	3683	10.714	5.1	0.544
Fetzeren	AR	1005	735 900	241 015	7671	16.571	5.1	0.519
Stein	SG	1020	733 655	228 725	8562	16.714	4.5	0.585
Feusisberg	SZ	865	697 570	224 390	8824	17.143	5.0	0.451
Bannholz	SZ	947	704 130	221 425	10917	15.714	4.8	0.481
Landscheidi	AR	935	734 638	240 576	11325	15.714	4.8	0.316
Feldmoos	SG	934	731 175	232 095	14739	16.571	4.9	0.521
Munzenriet	SG	1185	744 845	229 390	43397	16.714	4.8	0.403

Experimental design

Hand pollinations and seed measurements

In June 2000, we randomly selected twelve flowering *L. flos-cuculi* plants in the 19 parental populations and defined them as parental plants. We performed the first crosses (resulting in the F1) by pollinating 228 parental plants in two different ways. On each plant we performed a geitonogamous pollination (=inbred cross) between two flowers of the same plant, and an allogamous pollination (=outbred cross) between two flowers of two different plants (5-10 m away) from the same population (Figure 1). Pollinated flowers were protected by a nylon bag to avoid any external pollen contamination. After fruit maturation we collected mature capsules, placed them separately in paper bags and dried them at room temperature for 10 days.

We then counted the number of fully developed seeds (F1 seeds). We considered brown-black and round seeds as 'developed' and orange and square-edged smooth seeds as 'not developed'. We weighed all developed seeds in bulk for each capsule, and calculated individual seed mass dividing by the number of developed seeds.

In December 2000, we sowed up to 30 developed seeds per capsule in three batches of 10 seeds per 3.5 x 3.5 x 4.5 cm in multipots filled with BF4 substrate (Tref de Baat®, Coevorden, The Netherlands). We estimated germination percentage for each fruit five weeks after sowing.

In January 2001, we randomly selected two seedlings per family and transplanted them into individual 9 x 9 x 9 cm pots filled with the same substrate. We measured plant fitness (F1 plants; see next section for fitness measures) in July 2001, after fruit maturation.

Between March and April 2003 we carried out the second round of crosses (F2). We performed one inbred cross and one outbred cross per F1 plant, and in total pollinated 326 F1 plants. We applied the same procedure as described above for collecting (June and July 2003), counting and weighing F2 fruits and F2 seeds. We measured adult plant fitness (F1 adult plants) in September 2003.

In January 2004, we sowed F2 seeds, again following the same procedure as for the previous generation, and measured germination percentage for the F2 seeds.

Between March and April 2004 we performed the third generation of crosses (F3). We repeated the same design to obtain the third round of plants (F3). We performed one inbred cross and one outbred cross per F2 plant, and in total pollinated 560 F2 plants. We applied the same procedure as described above for collecting (June and July 2004), counting and weighing F3 capsules and F3 seeds. In September 2004 we measured adult plant fitness (F2 adult plants).

In January 2005, we sowed F3 seeds, and recorded germination percentage after five weeks. In September 2005, after fruit maturation, we measured adult plant fitness (326 F3 plants). Finally, for each of the 19 populations we had obtained three different generations of plants, belonging to eight different treatment lines resulting from three successive crosses on the parental, F1, and F2 plants.

To assess inbreeding levels of plants in our different treatment lines we considered that one generation of self-pollination sets the inbreeding coefficient f to 0.5 for the offspring. After two and three consecutive self-pollinations f reaches a value of 0.75 and 0.875, respectively, according to the formula $f_{(g+1)} = [(1+f_{(g)})/2]$, where g denotes a generation (Maynard Smith 1989).

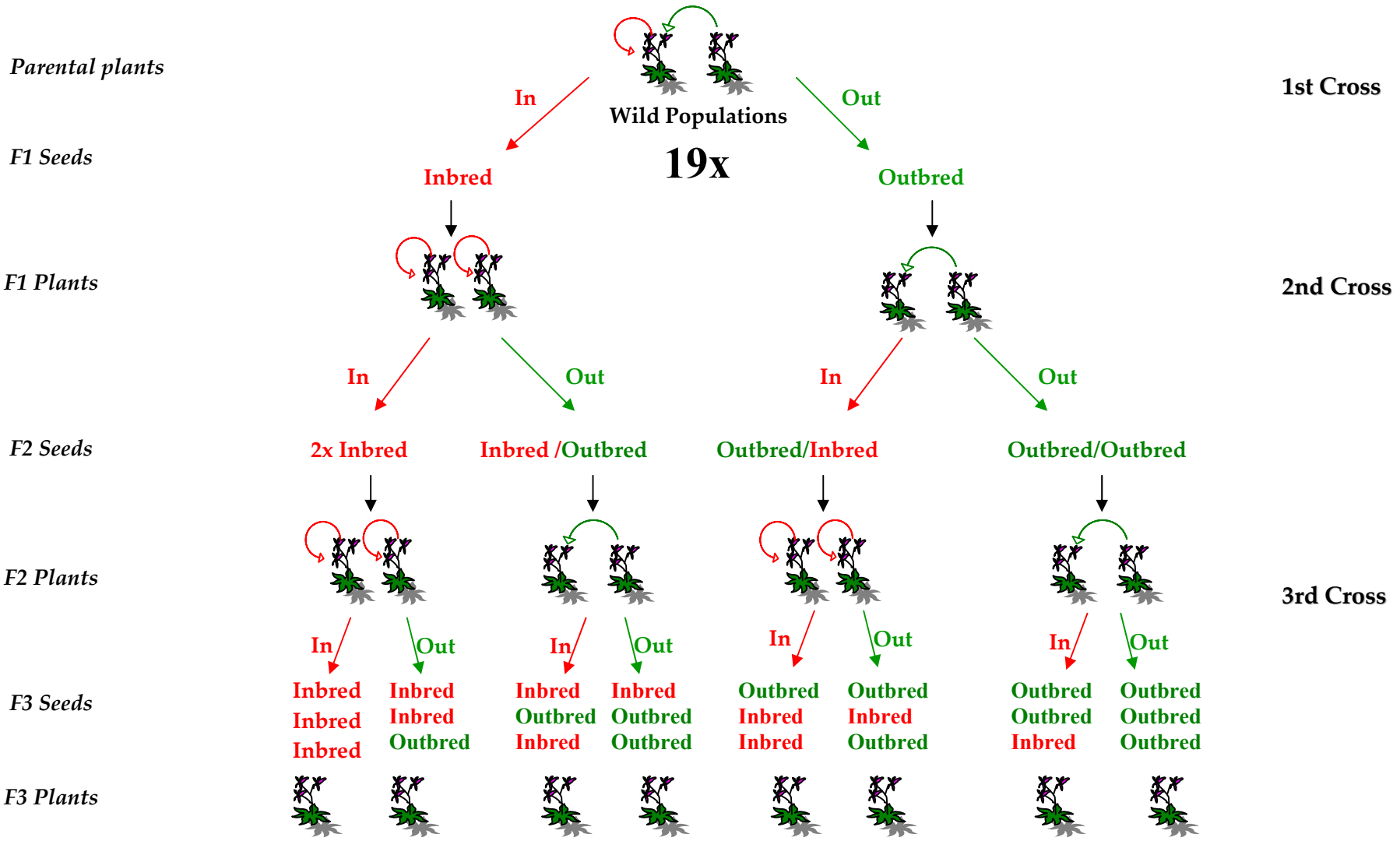
Parental plants
F1 Seeds
F1 Plants
F2 Seeds
F2 Plants
F3 Seeds
F3 Plants
Wild Populations
19x
In
Out
Inbred
Outbred
1st Cross
2nd Cross
3rd Cross
2x Inbred
Inbred /Outbred
Outbred/Inbred
Outbred/Outbred
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Figure 1: Design of the three-generations selfing and outcrossing experiment with plants of 19 populations of *Lychnis flos-cuculi*. Inbred crosses are crosses between two flowers of the same plant and outbred crosses are crosses between two flowers of different plants belonging to the same population.

Adult Fitness measurements

We assessed plant fitness considering vegetative and reproductive components. As vegetative measures we counted the number of vegetative rosettes. To evaluate reproductive fitness we recorded whether plants were flowering, and the number of flowering stems and of flowers per plant.

Cumulative fitness

To assess offspring plant fitness for the whole life-cycle we calculated cumulative fitness. As plant survival was 100%, we multiplied the number of fully developed seeds produced per fruit, germination percentage, and the number of flowers per plant per treatment and per generation for each of the original maternal plants:

Ψ_{F1} = Number of Seeds F1 * Germination percentage F1 * Number of Flowers F1

Ψ_{F2} = Number of Seeds F2 * Germination percentage F2 * Number of Flowers F2

Ψ_{F3} = Number of Seeds F3 * Germination percentage F3 * Number of Flowers F3

Similarly, to assess offspring fitness over the three generations we calculated total cumulative fitness for each treatment line and for each of the original maternal plants as:

$\Psi_{Tot} = \Psi_{F1} * \Psi_{F2} * \Psi_{F3}$

Genetic Load

We used cumulative fitness to estimate genetic load. For each population we obtained genetic load as the negative slope of the regression line between parental inbreeding coefficient (f) and log-transformed offspring fitness (Willi et al. 2005). In two cases, for which the estimate was slightly negative, we set it to 0.

Data Analysis

We analysed the fitness effects of characteristics of the populations of origin, of treatments, and of their interactions with hierarchical analysis of covariance (ANCOVA, type I sum of squares) with the statistical package S-PLUS (Versions 6.1/6.2, 2002 Insightful Corp., Seattle, USA). We used stepwise model selection to remove non-significant covariates from the model. We used experimental inbreeding coefficients f as a covariate in our model. Because it is not guaranteed that one generation of experimental outbred crosses completely resets f to 0, we only considered lines which ended with uninterrupted lines of inbreeding (3x inbred, out and 2x inbred, 2x outbred and 1x inbred; Figure 1) and the control of three subsequent outcrosses. We entered these treatment lines as factor "Treatment" in the model. We tested allelic richness and inbreeding coefficient of populations of origin against remaining variation among populations, and population, f, and treatment, against the population x treatment interaction (Table 2).

Table 2: Model of hierarchical analysis of covariance (ANCOVA) for assessing effects of habitat fragmentation, crossing treatments, and their interactions on *Lychnis flos-cuculi* offspring fitness. For F1 analyses, Inbreeding and Treatment are identical. For cumulative fitness analyses residual variation equals the population x Treatment interaction.

	Offspring fitness	
Source of variation	**Mean squares**	**Variance ratios (F values)**
Population size	$ms_{\text{Pop size}}$	$ms_{\text{Pop size}} / ms_{\text{Pop}}$
Allelic richness (AR)	ms_{AR}	$ms_{\text{AR}} / ms_{\text{Pop}}$
Inbreeding coefficient (Fis)	ms_{Fis}	$ms_{\text{Fis}} / ms_{\text{Pop}}$
Population	ms_{Pop}	$ms_{\text{Pop}} / ms_{\text{Pop x treatm}}$
Experimental inbreeding coefficient f	ms_{Inb}	$ms_{\text{Inb}} / ms_{\text{Pop x treatm}}$
Treatment	ms_{Treatm}	$ms_{\text{Treatm}} / ms_{\text{Pop x treatm}}$
Population size x Inbreeding	$ms_{\text{Pop size x Inb}}$	$ms_{\text{Pop size x Inb}} / ms_{\text{Pop x treatm}}$
AR x Inbreeding	$ms_{\text{AR x Inb}}$	$ms_{\text{AR x Inb}} / ms_{\text{Pop x treatm}}$
Fis x Inbreeding	$ms_{\text{Fis x Inb}}$	$ms_{\text{Fis x Inb}} / ms_{\text{Pop x treatm}}$
Population size x Treatment	$ms_{\text{Pop size x treatm}}$	$ms_{\text{Pop size x treatm}} / ms_{\text{Pop x treatm}}$
AR x Treatment	$ms_{\text{AR x treatm}}$	$ms_{\text{AR x treatm}} / ms_{\text{Pop x treatm}}$
Fis x Treatment	$ms_{\text{Fis x treatm}}$	$ms_{\text{Fis x treatm}} / ms_{\text{Pop x treatm}}$
Population x Treatment	$ms_{\text{Pop x treatm}}$	$ms_{\text{Pop x treatm}} / ms_{\text{Residuals}}$
Residuals (=Fruits)	$ms_{\text{Residuals}}$	

Results

Generation F1

Plants from populations with higher allelic richness and lower inbreeding coefficient F_{is} produced more F1 seeds regardless of the inbreeding treatment. Consequently, cumulative fitness of F1 plants was higher for populations of higher allelic richness and lower inbreeding coefficient F_{is} (Figure 2; Appendix 1A). The percentage of germination of seeds resulting from inbred crosses was 10.5 % lower than the one of seeds from outbred crosses (Figure 3)

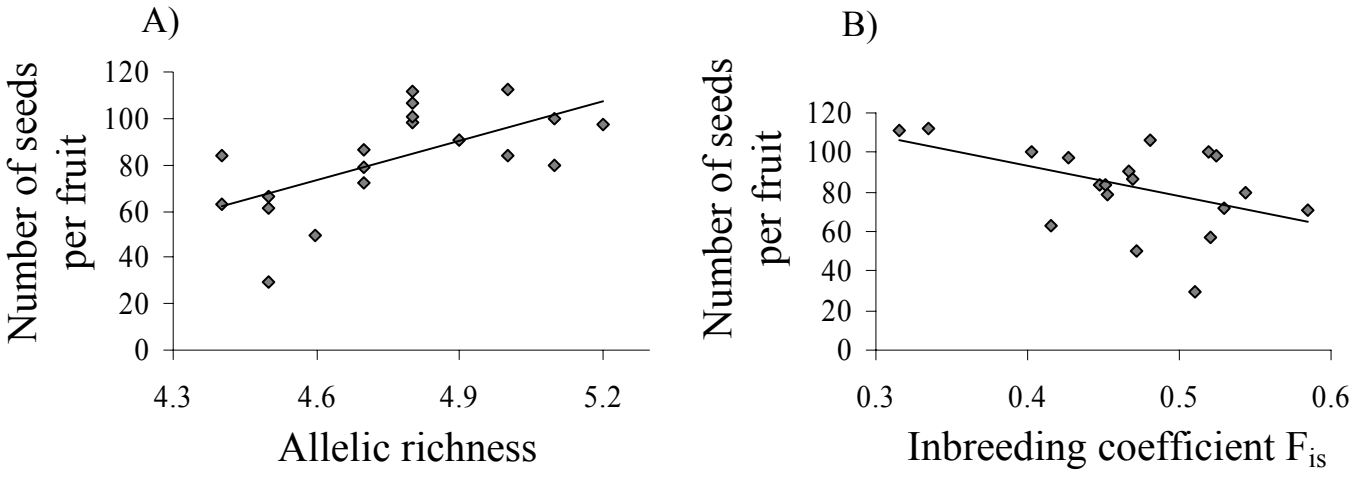

Figure 2: Influence of allelic richness A) and inbreeding coefficient F_{is} B) of populations of origin on the number of developed seeds per fruit in the F1 generation of *Lychnis flos-cuculi* (ANCOVA, $p<0.05$).

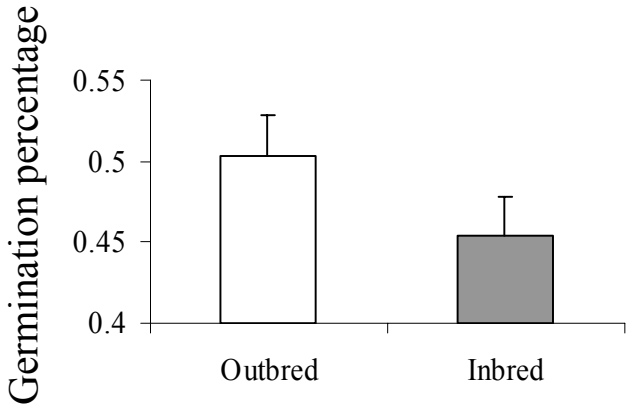

Figure 3: Effects of experimental inbreeding on germination percentage of F1 seeds of *Lychnis flos-cuculi* (ANCOVA, $p<0.01$).

Generation F2

After experimental inbreeding, inbreeding depression in cumulative fitness was stronger for plants from smaller than from larger populations of origin (Figure 4).

F2 seeds were not affected by characteristics of the population of origin anymore. The main effect of experimental inbreeding was significant for F2 cumulative fitness (Figure 5a; Appendix 1B) and we observed a reduction of cumulative fitness of 20.43% and of 58.64% after one and two experimental selfings, respectively. However, fruits originating from more homozygous populations had more developed seeds when at least one outbred cross had been performed, i.e when f was 0 or 0.5 ($p = 0.05$; Figure 5b).

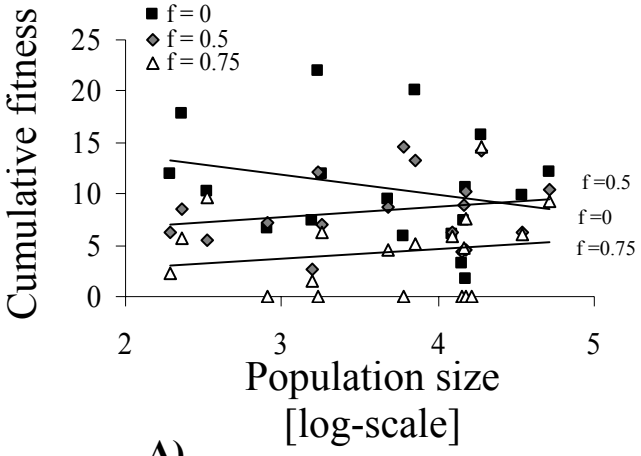

Figure 4: Larger populations of *Lychnis flos-cuculi* showed less variation in cumulative F2 fitness with varying degree of experimental inbreeding. Outbred crosses were more beneficial for F2 fitness of plants originating from smaller populations (ANCOVA, $p < 0.05$).

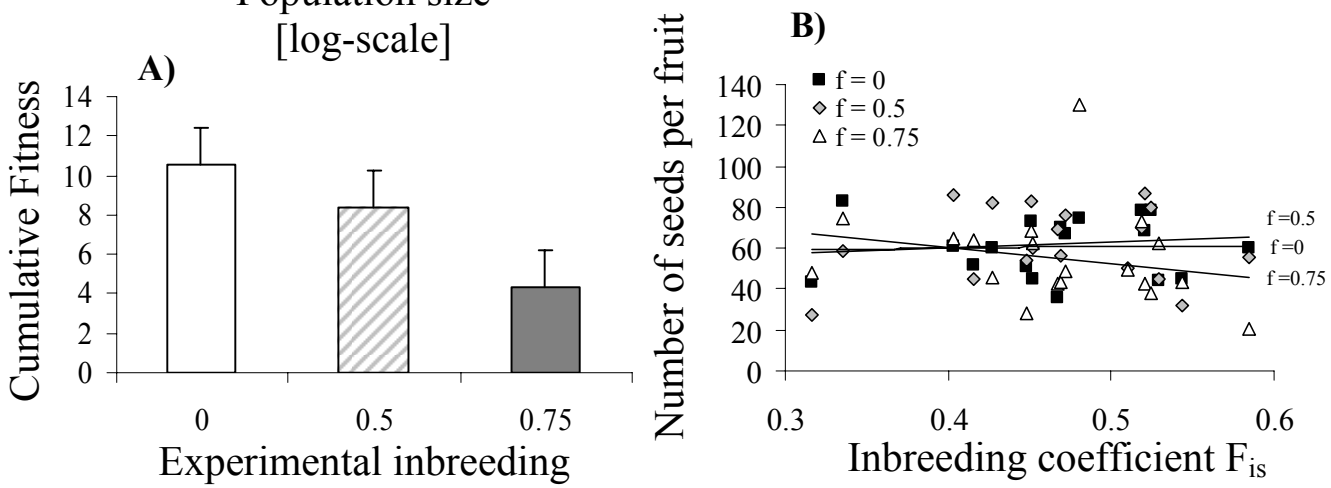

Figure 5: A) Inbreeding decreased F2 cumulative fitness after two generations of experimental selfing of *Lychnis flos-cuculi* plants (ANCOVA, $p<0.001$).
B) Two generations of experimental selfing increased F2 seed production for plants originating from less inbred populations and reduced it for plants from more inbred populations ($p = 0.05$).

Generation F3

Experimental inbreeding significantly reduced F3 seed germination percentage (Appendix 1C; $p<0.05$). Moreover, F3 seeds originating from larger populations had a higher germination percentage (Figure 6). More inbred offspring lines from populations with lower allelic richness tended to have increased cumulative fitness, as expected when inbreeding load is purged (Allelic richness x inbreeding coefficient f; Appendix 1C; $p<0.1$; Figure 7).

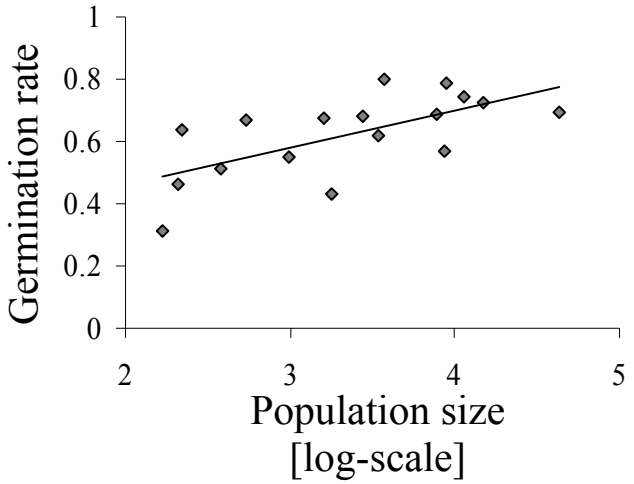

Figure 6: Plants from larger populations of *Lychnis flos-cuculi* produced F3 seeds with higher germination percentage regardless of the experimental crossing treatment (ANCOVA, $p < 0.01$).

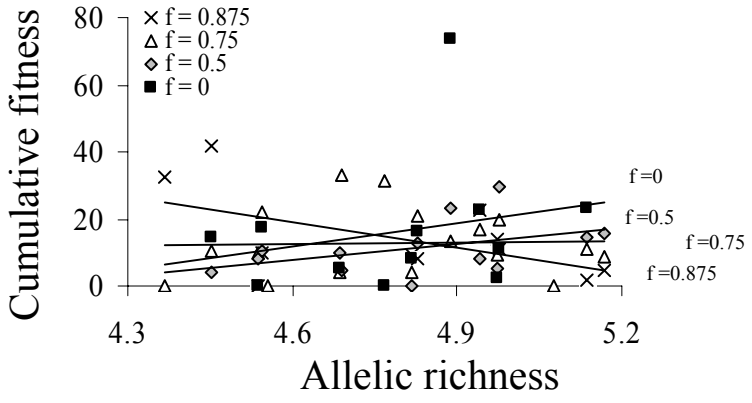

Figure 7: Effects of the interaction between inbreeding treatment and allelic richness of *Lychnis flos-cuculi* populations on F3 cumulative fitness (ANCOVA, $p<0.1$). Inbreeding depression was reduced for more inbred lines originating from less variable populations, suggesting purging of their genetic load.

Cumulative fitness over all generations

Finally, over all three generations cumulative fitness differed between populations of origin and decreased with increased experimental inbreeding (Figure 8; $p<0.05$).

Genetic load of the populations ranged from 0 to 2.28. It was independent of F_{is} and size of populations of origin, but significantly increased with increasing allelic richness of the population of origin ($p=0.05$; Figure 9).

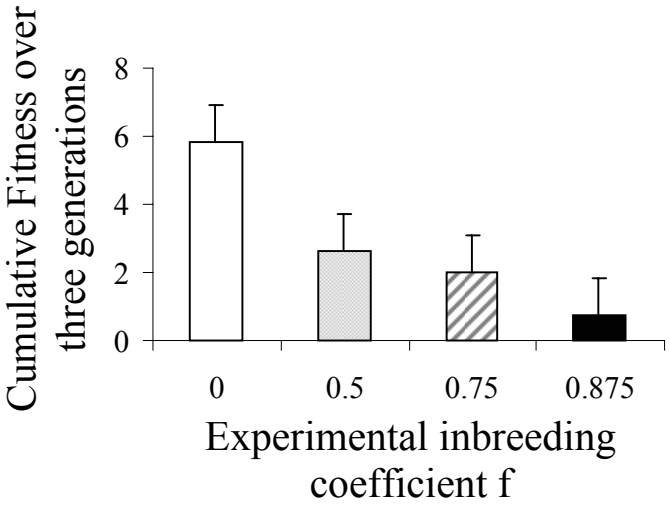

Figure 8: Cumulative fitness over three generations decreased with increasing experimental inbreeding coefficient f in the experiment with plants of *Lychnis flos-cuculi* (ANCOVA, $p<0.05$).

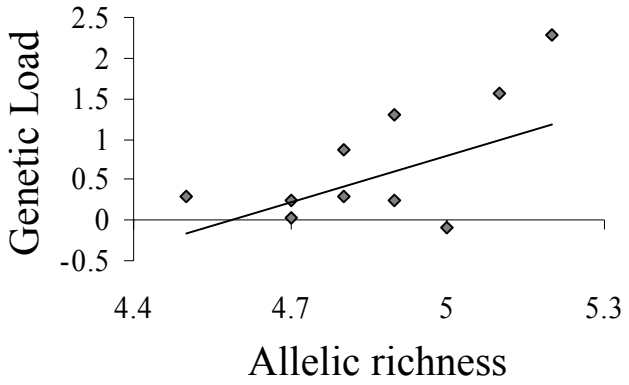

Figure 9: Genetic load was significantly reduced in populations with lower allelic richness. It suggests a reduction in inbreeding depression in smaller populations of *Lychnis flos-cuculi* due to purging (Linear regression, $p=0.05$).

Discussion

Allee effects

Our experimental conditions in the greenhouse allowed us to show that the observed differences between plants originating from different populations were due to genetic, and not due to environmental, differences. These differences in plant fitness cannot be explained by the presence of maternal carry-over effects (Roach and Wulff 1987, Oostermeijer et al. 1994, Schmid and Dolt 1994) because we did not observe any correlations between seed mass and germination or seedling mortality (Oostermeijer et al. 1994, Fischer et al. 2000). Moreover, maternal effects are usually expressed during the first stages of the life cycle (Roach and Wulff 1987). The poorer performance of plants from smaller populations remained apparent in the F1.

We detected reduced fitness of plants originating from smaller populations. Number of F1 seeds and F1 cumulative fitness were lower with lower allelic richness of the population of origin and F3 seedlings from smaller populations had a lower germination percentage. Moreover, F1 seed production and plant fitness were reduced with higher inbreeding coefficient F_{is} of the population of origin. These results extend earlier reports of Allee effects (Fischer et al. 2000, Hackney and McGraw 2001, Galeuchet et al. 2005b, Reed 2005), because they were obtained across several experimental crossing treatments, and maintained for three generations. These results also confirm that we really did our inbreeding study with plants representing the relevant genetic background.

Inbreeding depression

Fitness of *L. flos-cuculi* plants decreased with increasing degree of experimental inbreeding. Inbreeding depression was not expressed similarly in all stages of the life cycle and some fitness components were only affected after two generations of inbreeding. Corresponding to the results of Hauser and Loeschcke (1995), we found that germination was affected after one generation of experimental self-pollination. Contrasting with the previous results, seed production was affected by inbreeding only when two successive inbred crosses were performed.

Our results indicate that the still common species *L. flos-cuculi* suffers from inbreeding depression after one or several generations of inbreeding and depending on generation, different fitness traits are affected, which might greatly threaten population viability in a fragmented landscape.

Purging

The reduction of plant cumulative F2 fitness by inbreeding was stronger for plants from smaller populations, and inbreeding depression was also stronger for F2 plants from populations with higher F_{is}. These patterns are the opposite of the one expected if purging of inbreeding load had played a role, because there, inbreeding depression should have been reduced in small inbred populations (Hauser and Loeschcke 1994, Carr and Dudash 1997, Byers and Waller 1999). Thus, our results suggest that two generations of inbreeding are not sufficient for *Lychnis flos-cuculi* to purge the inbreeding load of smaller, less genetically variable populations.

In contrast, cumulative F3 fitness was affected by the interaction between allelic richness and experimental inbreeding in a way that less genetically variable populations had reduced inbreeding depression. Moreover, genetic load over all three generations was reduced for populations of lower allelic richness. These latter results suggest that some purging of inbreeding load is nevertheless taking place after three generations of inbreeding.

Conclusion

We conclude that inbreeding depression largely affects the still common *Lychnis flos-cuculi.* Inbreeding depression reduced offspring fitness at all stages of the life cycle. Moreover, inbreeding load is not rapidly purged from small inbred populations. Consequently, plant fitness is reduced for plants in such populations. To counteract effects of inbreeding depression on small and isolated populations of *Lychnis flos-cuculi,* population sizes need to be increased, and gene flow between populations promoted, at least as far as outbreeding depression does not become a problem.

Acknowledgements

We thank David Galeuchet for his work with the first cross generation, the owners and tenants of our field sites for allowing us to access these agriculturally used study sites, and Bianca and Gustav Ehrle for skillful care for our plants in the glasshouse. This research was supported by the Swiss National Science Foundation (Grants no. 31-56809.99 and 31-67876.02 to MF).

Appendix 1A

ANCOVA results for effects of population characteristics and experimental inbreeding on number and germination percentage of F1 seeds and cumulative F1 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F1

	Number of seeds				**Germination rate**				**Cumulative Fitness**			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**Ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Population size	1	236.85	0.0517	0.823	1	0.0221	0.1677	0.688	1	479'605	0.4300	0.522
Allelic richness (AR)	1	33675.58	7.3551	0.015*	1	0.2100	1.5952	0.226	1	5'816'840	5.2155	0.037*
Inbreeding coefficient (F_{is})	1	25588.9	5.5889	0.031*	1	0.2637	2.0031	0.177	1	6'459'239	5.7915	0.029*
Population	16	4578.56	2.1413	0.007**	15	0.1316	4.3362	<0.001***	15	1'115'292	0.9725	0.521
Experimental inbreeding coefficient f	1	11398.53	1.5459	0.233	1	0.1699	9.0637	0.009**	1	1'279'769	1.1159	0.308
Population size x inbreeding coeff. f	1	1998.65	0.2711	0.61	1	0.0126	0.6715	0.425	1	85'212	0.0743	0.789
AR x inbreeding coefficient f	1	2731.21	0.3704	0.552	1	0.0001	0.0044	0.948	1	197'493	0.1722	0.684
F_{is} x inbreeding coefficient f	1	8699.58	1.1798	0.295	1	0.0065	0.3456	0.565	1	2'026'864	1.7673	0.204
Population x treatment	15	7373.56	3.4485	<0.001***	15	0.0187	0.6177	0.859	15	1'146'870		
Residuals (=Fruits)	270	2138.2			241	0.0304						

Appendix 1B

ANCOVA results for F2 effects of population characteristics and experimental inbreeding on number and germination percentage of F2 seeds, and cumulative F2 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F2

	Number of seeds				Germination rate				Cumulative Fitness			
Source of variation	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Population size	1	1503.06	0.390	0.542	1	0.002	0.017	0.898	1	554	0.001	0.970
Allelic richness (AR)	1	11140.77	2.889	0.110	1	0.055	0.524	0.480	1	878813	2.356	0.146
Inbreeding coefficient (F_{is})	1	393.98	0.102	0.754	1	0.005	0.049	0.827	1	76220	0.204	0.658
Population	15	3856.93	2.054	0.011*	15	0.105	1.495	0.104	15	373061	3.567	0.002**
Experimental inbreeding coefficient f	1	4686.65	3.325	0.079	1	0.058	0.697	0.411	1	2889365	27.63	<0.001***
Treatment	1	6939.38	4.923	0.035*	1	0.052	0.618	0.438	1	379031	3.624	0.067
Population size x inbreeding coeff. f	1	159.38	0.113	0.739	1	0.035	0.421	0.522	1	560474	5.359	0.028*
AR x inbreeding coefficient f	1	261.78	0.186	0.670	1	0.007	0.085	0.773	1	87232	0.834	0.369
F_{is} x inbreeding coefficient f	1	1583.41	1.123	0.298	1	0.108	1.292	0.265	1	169499	1.620	0.213
Population size x treatment	1	666.38	0.473	0.497	1	0.000	0.002	0.968	1	50274	0.481	0.494
AR x treatment	1	8.08	0.006	0.940	1	0.046	0.553	0.463	1	93818	0.897	0.352
F_{is} x treatment	1	5726.46	4.063	0.054*	1	0.033	0.393	0.536	1	233710	2.235	0.146
Population x treatment	28	1409.46	0.751	0.819	28	0.084	1.188	0.238	28	104576		
Residuals (=Fruits)	384	1877.69			355	0.070						

Appendix 1C

ANCOVA results for effects of population characteristics and experimental inbreeding on number and germination percentage of F3 seeds, and cumulative F3 fitness of *Lychnis flos-cuculi* offspring. We present degrees of freedom (df), mean squares (ms), F, and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Generation F3

	Number of seeds				**Germination rate**				**Cumulative Fitness**			
Source of variation	**df**	**ms**	**F**	**p**	**Df**	**ms**	**F**	**p**	**df**	**ms**	**F**	**p**
Population size	1	2747.03	0.495	0.494	1	0.717	11.03	0.006**	1	1’163’291	0.552	0.471
Allelic richness (AR)	1	4797.94	0.864	0.369	1	0.228	3.510	0.084	1	68’307	0.032	0.860
Inbreeding coefficient (F_{is})	1	3.493	0.001	0.980	1	0.031	0.483	0.499	1	232’728	0.110	0.745
Population	13	5550.45	2.641	0.003*	13	0.065	1.693	0.070	13	2’108’048	1.354	0.251
Experimental inbreeding coefficient f	1	140.84	0.073	0.789	1	0.200	6.736	0.016*	1	134’035	0.086	0.772
Treatment	2	3325.7	1.732	0.197	2	0.093	3.122	0.062+	2	1’254’514	0.806	0.458
Popsize x inbreeding coefficient f	1	122.41	0.063	0.802	1	0.060	2.034	0.166	1	1’357’237	0.872	0.360
AR x inbreeding coefficient f	1	116.38	0.061	0.808	1	0.006	0.191	0.666	1	5’476’092	3.518	0.073^{+}
F_{is} x inbreeding coefficient f	1	5.198	0.002	0.959	1	0.066	2.213	0.149	1	5’017’845	3.223	0.085
Population size x treatment	2	123.34	0.064	0.938	2	0.029	0.963	0.395	2	118’467	0.076	0.927
AR x treatment	2	247.1	0.129	0.880	2	0.093	3.137	0.061+	2	1’066’284	0.685	0.514
F_{is} x treatment	2	9166.86	4.774	0.018*	2	0.030	1.024	0.374	2	1’729’169	1.111	0.346
Population x treatment	25	1920.18	0.914	0.586	25	0.030	0.773	0.769	24	1’556’524		
Residuals (=Fruits)	124	2101.58			126	0.038						

Chapter 5

Offspring Fitness after Inter-Population Crosses of the Common *Lychnis flos-cuculi* in Relation to Geographic, Genetic and Ecological Distances between Populations

With Gillianne Bowman and Markus Fischer

Abstract

We evaluated the consequences of between-population crosses for plants of 13 populations of the common but declining *Lychnis flos-cuculi*. Previous information on molecular diversity and ecological conditions allowed us to study the roles of genetic diversity, and of genetic and ecological, along with geographical, distances between populations for offspring fitness. We crossed 7 plants of each of the 13 populations with a plant from: 1/ the same population, 2/ a small, 3/ a large, 4/ an ecologically similar and 5/ an ecologically dissimilar population to obtain the F1 generation. We grew plants of this F1 generation until flowering both in the greenhouse and in the field populations of origin. Moreover, we used the greenhouse plants to perform F2 and backcross crosses. Offspring of crosses between populations had higher vegetative and reproductive cumulative fitness in the F1 both in the greenhouse and in the field, indicating heterosis rather than breaking-up of local adaptation. F1 performance also increased with larger geographic and ecological distances between parental populations. Although the heterosis effect was still positive in the F2 and backcrosses, it was not significant any more. Within-population crosses tended to be more beneficial for populations with lower allelic richness for most fitness traits and generations. Nevertheless, overall our study suggests that heterosis after between population crosses outweighs potential negative consequences.

Introduction

Habitat fragmentation reduces population size and gene flow between populations. Small populations have reduced genetic diversity because of inbreeding and genetic drift (Barrett and Kohn 1991, Ellstrand and Elam 1993). Linked with the loss of genetic diversity and increased inbreeding, habitat fragmentation is also often associated with a reduction in plant fitness in small populations (Young et al. 1996, Fischer and Diethart 1998, Kolb 2005), the so-called Allee effect (Stephens *et al.* 1999). To counteract these effects, genetic rescue by artificial gene flow may be a valuable tool (Ingvarsson 2001, Tallmon et al. 2004, Hedrick 2005). Recreating gene flow disrupted by landscape fragmentation introduces new alleles to threatened populations, thus reducing inbreeding and increasing fitness of crosses between different populations relative to within-population crosses (Edmans 1999, Vergeer et al. 2004, Willi and Fischer 2005).

However, crosses between populations can also lead to outbreeding depression. Crosses between genetically distant populations can break-up co-adapted gene complexes (Lynch 1991). Alleles can behave differently depending on the genomic context they belong to and beneficial epistatic genetic effects can be disrupted. Moreover, plant populations often show strong local adaptation in relation to differences in local ecological conditions (Schmitt and Gamble 1990, Linhart and Grant 1996, Joshi et al. 2001, Hufford and Mazer 2003, Kawecki 2004, Vergnerie et al. in prep). Therefore, the effects of mixing of gene pools can also be detrimental. Indeed, break-up of local adaptation has been found in several studies on inter-population crosses (Waser 1993b, Fischer and Matthies 1997).

Differences between populations play an important role in predicting consequences of crosses between populations. These differences can be estimated in different ways. First, larger geographical distances between populations may be proxies for larger genetic and environmental differences. Second, selectively neutral molecular genetic distances directly indicate how closely related populations are. Third, quantitative genetic distances indicate how genetically similar populations are in ecologically relevant traits. Fourth, distances in ecological conditions may be proxies for differences due to local adaptation.

To evaluate the consequences of local adaptation and fragmentation for fitness after inter-population crosses, we used the common but declining *Lychnis flos-cuculi.* We performed inter-population crosses among 13 populations differing in size and ecological

conditions and grew the resulting offspring in the greenhouse and in the parental natural populations. Moreover, we produced F2 and backcrosses to study longer-term consequences of inter-population crosses. We wanted to answer the following questions: 1- Is offspring fitness larger after crosses between populations than within populations and how does this change across generations? 2- Are benefits of between-population crosses higher for plants from smaller populations? 3- Do consequences of between-population crosses change with geographical, genetic, and ecological distances between populations?

Material & Methods

Study species

The ragged robin *Lychnis flos-cuculi* (= *Silene flos-cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun) (Caryophyllaceae) is common throughout most of Europe. It grows in sunny, wet-to-moist habitats such as fens and wet meadows and can be found from the plain to the montane level.

This rosette forming, perennial herb reproduces both sexually and clonally and produces several flowering stems, bearing up to 50 flowers in dichasial inflorescences. The flowers are protandrous, but while plants are mainly outcrossed (Biere 1996), selfing still occurs. The main pollinators are Diptera and Hymenoptera (*Bumbus*) (Vejsnæs and Høvsgaad 1990). In our study area, plants flower mainly in June-July and fruits mature in August. Fruit capsules contain up to 200 seeds, which are dispersed principally around the maternal plant by vibration of the stiffened stalk. The seeds are able to germinate in autumn and in spring (Biere 1991).

Although still common, populations of *L. flos-cuculi* have been declining in the last decades due to changes in agriculture and urbanisation, and therefore it is a good model for studying the effects of habitat fragmentation. From an applied point of view, mixing gene pools of *L. flos-cuculi* is interesting because it is often used in restoration measures in Switzerland such as sown wild flower strips in the agricultural landscape (Lehmann *et al.* 2000).

Plant origin

Our 13 populations of plant origin belong to a larger set of populations studied in a demographic study in North-Eastern Switzerland (Hoehn *et al*. in prep). In 2001, population size of the six small (<800) and seven large (>2500) populations ranged from 40 to 51 000 flowering stems (Table 1) and allelic richness of these populations varied from 4.46 to 4.98. To characterize the habitat of each population, we surveyed the fen vegetation in July 2002. We recorded the identity and ground cover of all higher plant species present in a two m^2-plot situated at a random position within the *L. flos-cuculi* population. From the species composition weighted by ground cover we calculated mean ecological indicator values after Landolt (1977) for each habitat. For this study we consider the indicator values describing the ecological gradients most relevant for *L. flos-cuculi*, i.e. the light (L), moisture (F), nutrient (N) and soil acidity (R) indicator values. On scales from 1 (low) to 5 (high) they ranged from 2.95 to 3.94 for F, from 1.67 to 2.89 for N, from 1.88 to 2.99 for L, and from 1.65 to 3.05 for R. Mean indicator values were not correlated with population size ($n = 15$, F: $r = -0.11$, $p = 0.7$; N: $r = 0.00$, $p = 0.99$; L: $r = 0.24$, $p = 0.39$; R: $r = -0.28$, $p = 0.29$). The variation in the ecological conditions between its populations makes *L. flos-cuculi* a good study species for local adaptation.

We assessed pairwise geographic, genetic, and ecological distances between the 13 populations. We calculated geographic distances from our field recordings of site coordinates with a GPS (Global Positioning System, Garmin, Olathe, Kansas, U.S.A.). We obtained molecular genetic distances between pairs of populations as F_{ST} and allelic richness for each population from a study of 8-18 plants per population with seven microsatellite markers (Galeuchet et al. 2005a). We calculated mean quantitative trait distances Q_{st} between pairs of populations (Merila and Crnokrak 2001) with the data from Galeuchet (2005b). We assessed ecological distance between pairs of populations as the absolute differences between population means of the F, N, L, and R indicator values to describe differences in habitat quality between parental populations.

Table 1: Characteristics of the 13 study sites and populations of *Lychnis flos-cuculi*. We give municipality (Swiss canton in parentheses), coordinates as in Swiss topographical maps, altitude, population size and mean ecological indicator values. Population size denotes the number of flowering stems in 2000. Populations were the 6 smallest (<800) and 7 largest (>2500) of a larger set used for other demographic and genetic studies. Mean ecological indicator values after Landolt (1977) are based on vegetation records in 2002 as explained in methods. We obtained allelic richness from a study of 8-18 plants per population with 7 microsatellite markers (Galeuchet, 2005b). We used Schlänggen (SZ) population for crosses in the greenhouse but we did not transplant plants to this site.

Site				Coordinates			Mean indicator value for			
	Canton	Population size	Altitude (m)	E	N	Allelic Richness	Light (L)	Moisture (F)	Nutrient (N)	Acidity (R)
Bühler	AR	40	940	751'865	250'165	4.77	2.91	3.94	2.49	2.91
Seilerzwecken	SZ	157	1330	697'355	209'930	4.46	2.51	3.56	2.82	2.77
Gäbris Wald	AR	192	1175	753'430	249'915	4.83	2.48	3.63	2.97	2.88
Allmeindswald	SG	330	1080	732'220	237'525	4.98	2.99	3.68	2.66	2.12
Schlänggen	SZ	500	900	705'586	213'240	4.63	1.88	2.95	1.67	1.86
Hasenried	SG	800	1171	740'520	233'400	4.69	2.53	3.78	2.55	3.05
Sulzel	SZ	2744	960	703'400	222'400	4.56	2.73	3.75	2.39	2.11
Bannholz	SZ	11893	950	704'130	221'425	4.91	2.87	3.23	2.89	2.59
Stein	SG	14688	950	733'655	228'725	4.54	2.68	3.58	2.6	1.87
Strandbad	SZ	16502	900	700’950	223’100	4.78	2.8	3.75	2.5	2.04
Feusisberg	SZ	19047	875	697'570	224'390	4.97	2.97	3.69	2.86	2.6
Feldmoos	SG	35000	890	731'175	232'095	4.94	2.73	3.61	2.59	2.37
Munzenriet	SG	51000	1130	744'845	229'390	4.82	2.85	3.72	2.47	1.65

Crosses

For our crosses, we started with two-year-old plants grown from seeds sampled in the field in July 2000 and sown in September 2000 in the greenhouse (Perret 2003b, Galeuchet et al. 2005b). We kept all plants above 0°C in winter and provided natural light, and water according to needs.

First generation (F1)

In spring 2003, we performed within- and between-population crosses with offspring of 13 natural populations. We produced four types of between-population crosses: with large, small, ecologically similar and ecologically dissimilar (according to ecological indicator values for light and moisture) populations. We used 107 plants to perform 410 crosses, resulting in 281 fruits (Figure 1A).

In August 2003, we counted the number of seeds and sowed up to 30 seeds per fruit in multi-pot trays with 15 seeds per 3 x 3 cm pot containing standard substrate (BF4, Tref de Baat, The Netherlands). We scored germination after four weeks. In September 2003, we randomly selected three plantlets per fruit and transplanted them individually to 10-cm-diameter pots filled with the same substrate. We kept them in a greenhouse (921 plants). We also transplanted four additional plantlets per fruit to 12 of our natural populations (991 plants), two to each parental population. In summer 2004, we monitored F1 plants in the greenhouse and in the field. We measured plant survival, the size and numbers of vegetative and reproductive parts, and we additionally assessed plant damage by pathogens and herbivores in the field.

Second generation (F2) and backcrosses to the maternal population (Ba)

In spring 2004, we performed 323 F1 x F1 crosses (287 fruits) to obtain F2 plants from within and between-population crosses (Figure 1A). For each population of origin, we crossed only F1 plants from the same of the five F1 cross types to obtain F2. The F1 within-population crosses were crossed among themselves for each population of origin

as control. We also performed 202 backcrosses of within-population F1 and of between-population F1 with the original maternal plants resulting in 172 fruits (Figure 1B).

In September 2004, we counted the resulting seeds and sowed up to 30 seeds per fruit in multi-pots trays with 15 seeds per 3 x 3 cm pots containing standard substrate. Again, we scored germination after four weeks. In October 2004, we randomly selected two plantlets per fruit and transplanted them in the same way in the greenhouse than the F1 (1294 plants). We additionally grew F2 and backcrosses in competition with *Anthoxanthum odoratum* (20 commercial seeds added during transplantation to 10-centimeter-diameter pots; 918 *L. flos-cuculi* plants). We used competition to test whether effects of between-populations crosses change with plant stress. F2 reproductive fitness decreased significantly with competition ($F_{1.334}$=233, $p<0.05$) in accordance with previous results (Galeuchet et al. 2005b). In summer 2005, we measured the number of rosettes and of flowers, and survival.

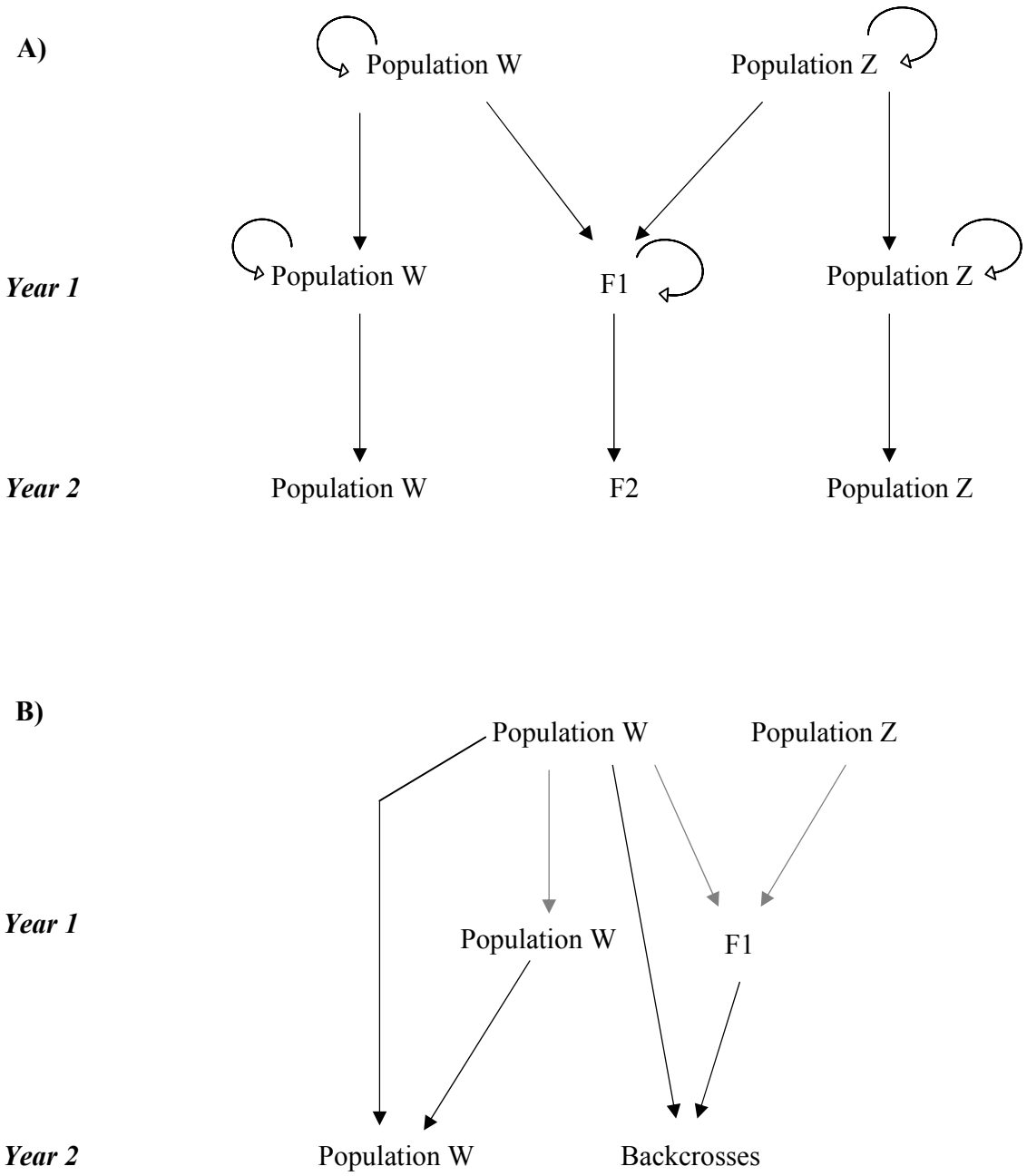

Figure 1: Schemes of the crosses performed in the greenhouse with plants from pairs of 13 natural populations of *Lychnis flos-cuculi*.

Data analysis

We used logistic regression to analyse binomial data (Survival) with the statistical package R. We divided mean deviances due to a factor by their appropriate error mean deviances, analogous to the calculation of F-values in ordinary analysis of variance (Payne *et al.* 1993). We analysed vegetative (number of seeds x germination rate x number of rosettes) and reproductive (number of seeds x germination rate x number of flowers) cumulative fitness, both square root transformed to meet the assumption of normality, with hierarchical analyses of covariance (ANCOVA) with the statistical software package JMP (Version 4.0.1, SAS Institute Inc. 2000, Cary, North Carolina 27513, USA.).

For the F1 in the greenhouse and in the field, we tested for effects of allelic richness of maternal population, population size and ecological indicator values (humidity, acidity, nutrient, light) to examine the role of habitat fragmentation characteristics on plant performances. We also tested effects of paternal population characteristics. We tested the effects of within- versus between-population crosses and of the four different between-population crosses to assess the consequences of different types of crosses. We tested effects of neutral and quantitative genetic distances between populations (pairwise F_{st} and Q_{st} values) and effects of geographic distances between populations and also the absolute difference between indicator values of maternal and paternal populations. Then we tested effects of the interaction between allelic richness of the maternal population with the treatments. We also tested for the effects of corresponding interactions of the allelic richness of paternal populations (see Table 2 for ANCOVA model). To analyse the performance of the F1 transplanted to the field, we added transplant site as source of variation and tested it against the residual (see Table 3 for ANCOVA model). To analyse the F2 and backcrosses, we used a similar but slightly reduced model (see Table 4 and 5 for ANCOVA model).

Results

Characteristics of parental populations

In the greenhouse, F1 offspring from maternal populations with higher allelic richness had higher vegetative (number of seeds x germination rate x number of vegetative rosettes) and reproductive (number of seeds x germination rate x number of flowers) fitness, indicating genetic Allee effects ($F_{1,7}$=8.7, $p<0.05$ and $F_{1,7}$=10.6, $p<0.05$ respectively; i.e. Figure 2).

For the F1 transplanted to the field, vegetative plant performance decreased significantly with increasing maternal population size ($F_{1,6}$=11.3, $p<0.05$). Plant performance also depended on several characteristics of the paternal population. Reproductive fitness increased with acidity indicator value of the paternal population ($F_{1,6}$=6.1, $p<0.05$). Survival increased with humidity ($F_{1,6}$=6.4, $p<0.05$) and light ($F_{1,6}$=8.5, $p<0.05$) indicator value, and decreased with nutrient indicator value ($F_{1,6}$=7.9, $p<0.05$) of the paternal population. Thus, characteristics of maternal and paternal populations explained variation in plant performance when transplanted to the field.

Residual variation among maternal and paternal families and populations was highly significant for most cumulative fitness measures and generations, confirming a strong genetic component of plant performance. At the same time, for all three fitness traits of the F1 in the field, the site of transplantation was a significant source of variation.

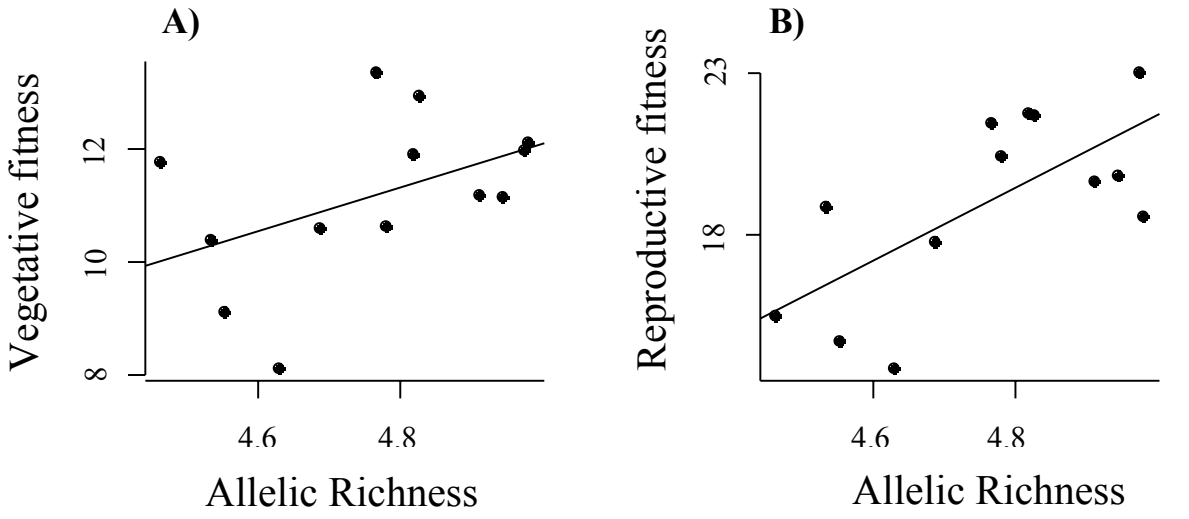

Figure 2: Allee effect in offspring of crosses within and between populations of *Lychnis flos-cuculi.* Positive effect of maternal population allelic richness on vegetative ($p<0.05$) (A) and reproductive ($p<0.05$) (B) cumulative fitness in F1 plants in the greenhouse, all crosses included. Levels of significance were obtained with hierarchical ANCOVA (Table 2). Vegetative and reproductive fitness denote the total number of offspring rosettes or flower resulting per original fruit after one generation.

Table 2: Analyses of covariance for vegetative and cumulative fitness of F1 plants in the greenhouse after crosses within and between populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Factors and covariates comprise a set of parental population characteristics, cross types (S=Small, L=Large, Si=Similar, Di=Dissimilar), and genetic, geographic, and ecological distances between these populations. Population denotes the 13 parental populations. We square root transformed the data to meet the assumption for normality. We present mean squares (ms), F and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (df = degrees of freedom).

►►

F1 fitness in the greenhouse	**Vegetative**				**Reproductive**			
Sources of variation	df	ms	F	p	df	ms	F	p
Maternal Pop. A.R.	1	390.7	8.67	*	1	2817.1	10.63	*
Maternal Pop. size	1	250.6	5.56	+	1	61.1	0.23	
Maternal Pop. Humidity (F)	1	99.1	2.20		1	552.7	2.08	
Maternal Pop. Acidity (R)	1	0.1	0.00		1	41.5	0.16	
Maternal Pop. Nutrient (N)	1	227.0	5.04	+	1	593.4	2.24	
Maternal Pop. Light (L)	1	23.1	0.51		1	145.8	0.55	
Maternal Population	7	45.0	0.45		7	265.1	0.79	
Maternal plants	86	99.8	14.89	***	86	336.9	50.30	***
Paternal Pop. A.R.	1	40.3	0.60		1	180.2	0.80	
Paternal Pop. Size	1	98.8	1.47		1	590.9	2.62	
Paternal Pop. Humidity (F)	1	0.7	0.01		1	49.7	0.22	
Paternal Pop. Acidity (R)	1	0.2	0.00		1	147.0	0.65	
Paternal Pop. Nutrient (N)	1	9.3	0.14		1	11.1	0.05	
Paternal Pop. Light (L)	1	77.6	1.16		1	553.3	2.45	
Paternal Population	7	67.2	1.33		7	225.8	1.30	
Paternal plant	93	50.3	7.51	***	93	173.9	25.97	***
Cross (W. vs B.)	1	310.2	8.21	**	1	1041.3	7.40	*
Cross Type (S, L, Si, Di)	3	121.6	3.22	*	3	283.4	2.01	
Geo. Dist	1	22.9	0.61		1	155.8	1.11	
F_{st}	1	6.1	0.16		1	19.1	0.14	
Mean Q_{st}	1	39.8	1.05		1	315.0	2.24	
Humidity (F) Dist.	1	21.7	0.57		1	1.4	0.01	
Acidity (R) Dist.	1	1.5	0.04		1	18.3	0.13	
Nutrient (N) Dist.	1	149.2	3.95	+	1	246.6	1.75	
Light (L) Dist.	1	184.3	4.88	*	1	898.6	6.39	*
Cross x Maternal Pop. A.R.	1	54.1	1.43		1	131.1	0.93	
Cross Type x Maternal Pop. A.R.	3	26.2	0.69		3	73.8	0.52	
Geo. Dist x Maternal Pop. A.R.	1	4.1	0.11		1	6.7	0.05	
F_{st} x Maternal Pop. A.R.	1	2.5	0.07		1	0.1	0.00	
Cross Type x Paternal Pop. A.R.	3	34.8	0.92		3	31.3	0.22	
Geo. Dist x Paternal Pop. A.R.	1	1.8	0.05		1	5.2	0.04	
F_{st} x Paternal Pop. A.R.	1	23.7	0.63		1	0.4	0.00	
Maternal Pop x Paternal Pop	22	37.8	5.64	***	23	140.7	21.01	***
Residual	631	6.7			617	6.7		

Within versus between population crosses

F1 in the greenhouse

F1 plants from between-population crosses had significantly higher vegetative ($F_{1,22}$=8.2, $p<0.01$) and reproductive ($F_{1,23}$=7.4, $p<0.05$) fitness in the greenhouse than plants from within-population crosses (Figure 3), showing a clear heterosis effect with all four between-population cross types.

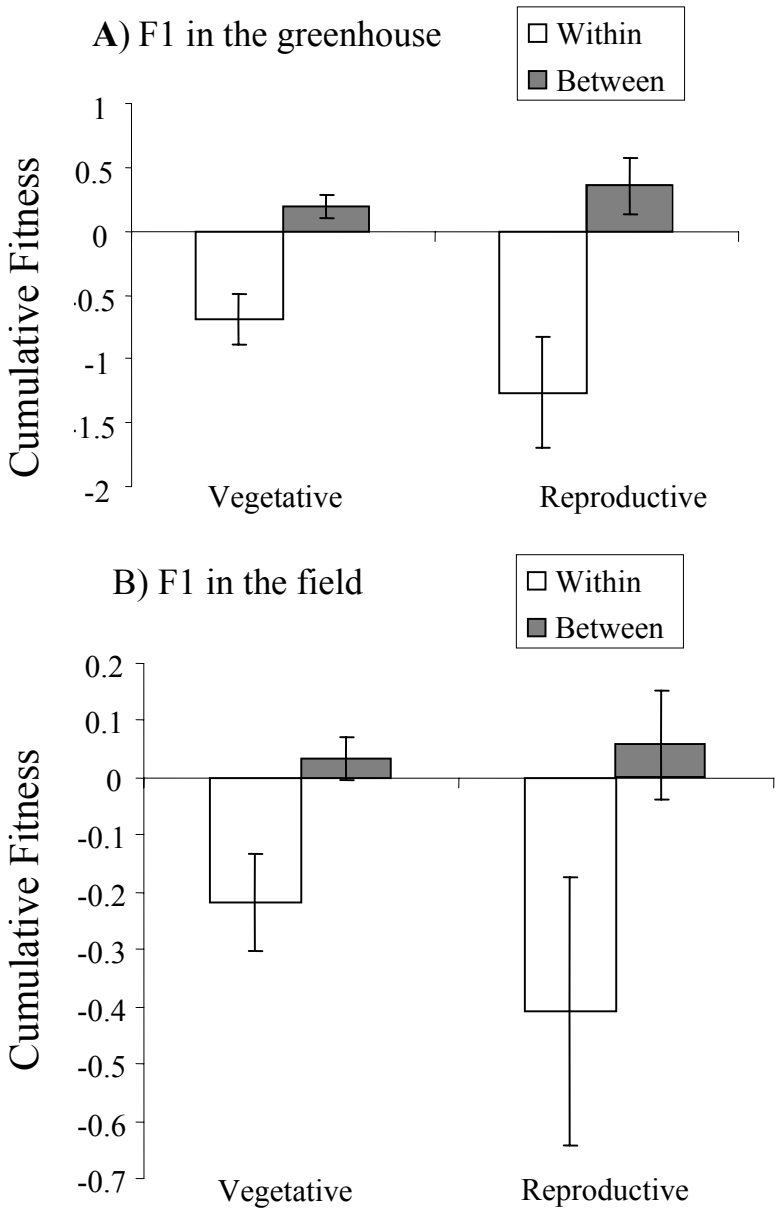

Figure 3: Means of residual fitness of the 13 natural populations of *Lychnis flos-cuculi* for offspring of within and between population crosses. F1 plants from between population crosses had significantly higher vegetative ($p<0.01$) and reproductive ($p<0.05$) fitness in the greenhouse (A) and higher vegetative ($p<0.1$) and reproductive ($p<0.001$) fitness in the field (B). Levels of significance were obtained with hierarchical ANCOVA (Table 2 and 3). Bars indicate one S.E.

Table 3: Analyses of covariance for survival, vegetative and cumulative fitness of F1 transplanted to the natural populations after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Factors and covariates comprise a set of parental population characteristics, cross types (S=Small, L=Large, Si=Similar, Di=Dissimilar), and genetic, geographic, and ecological distances between these populations. Population denotes the 13 parental populations. We square root transformed the data to meet the assumption for normality. We present mean squares (ms) or mean deviance changes (mdev), F and p values: + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

F1 fitness in the field	Vegetative				Reproductive				Survival			
Sources of variation	df	ms	F	p	df	ms	F	p	df	ms	F	p
Maternal Pop. A.R.	1	6.4	1.01		1	116.3	4.83	+	1	2.95	4.73	+
Maternal Pop size	1	72.1	11.27	*	1	17.5	0.73		1	0.02	0.04	
Maternal Pop. Humidity (F)	1	0.1	0.02		1	10.2	0.42		1	0.62	0.99	
Maternal Pop. Acidity (R)	1	0.0	0.01		1	16.2	0.67		1	3.17	5.07	+
Maternal Pop. Nutrient (N)	1	16.0	2.50		1	12.1	0.50		1	1.19	1.91	
Maternal Pop. Light (L)	1	4.6	0.72		1	127.4	5.30	+	1	0.97	1.55	
Maternal Population	6	6.4	0.98		6	24.1	0.83		5	0.62	3.17	*
Maternal plants	84	6.5	6.27	***	81	28.8	5.27	***	85	0.20	0.04	
Paternal Pop. A.R.	1	4.8	0.78		1	37.5	2.39		1	1.11	5.57	+
Paternal Pop Size	1	0.7	0.12		1	21.1	1.34		1	0.33	1.64	
Paternal Pop. Humidity (F)	1	0.2	0.04		1	0.2	0.01		1	1.28	6.42	*
Paternal Pop. Acidity (R)	1	4.5	0.72		1	95.7	6.08	*	1	0.02	0.09	
Paternal Pop. Nutrient (N)	1	1.8	0.29		1	0.0	0.00		1	1.58	7.94	*
Paternal Pop. Light (L)	1	7.8	1.26		1	26.0	1.65		1	1.68	8.46	*
Paternal Population	7	6.2	1.52		8	15.7	0.93		7	0.20	0.86	
Paternal plant	91	4.0	3.89	***	81	17.0	3.11	***	91	0.23	0.04	
Transplant Site	11	3.5	3.34	***	11	13.8	2.53	**	11	1.23	0.23	
Cross (W. vs B.)	1	14.8	4.10	+	1	38.5	8.53	***	1	0.30	1.28	
Cross Type (S, L, Si, Di)	3	2.0	0.56		3	0.9	0.20		3	0.08	0.36	
Geo. Dist	1	8.7	2.41		1	27.7	6.13	*	1	0.09	0.37	
F_{st}	1	5.7	1.58		1	11.8	2.62		1	0.01	0.06	
Mean Q_{st}	1	0.4	0.11		1	0.7	0.15		1	0.06	0.25	
Humidity (F) Dist.	1	17.2	4.76	*	1	23.8	5.27	+	1	0.73	3.12	+
Acidity (R) Dist.	1	9.8	2.73		1	26.1	5.78	*	1	0.34	1.44	
Nutrient (N) Dist.	1	3.3	0.91		1	0.6	0.12		1	0.24	1.01	
Light (L) Dist.	1	4.8	1.33		1	15.0	3.33		1	0.19	0.83	
Cross x Maternal Pop. A.R.	1	0.1	0.02		1	4.8	1.07		1	0.05	0.20	
Cross Type x Maternal Pop. A.R.	3	0.6	0.16		3	5.1	1.12		3	0.11	0.49	
Geo. Dist x Maternal Pop. A.R.	1	6.5	1.81		1	74.7	16.55	**	1	0.12	0.53	
Fst x Maternal Pop. A.R.	1	0.0	0.01		1	1.5	0.33		1	0.01	0.02	
Cross Type x Paternal Pop. A.R.	3	1.0	0.27		3	17.9	3.97	+	3	0.15	0.65	
Geo. Dist x Paternal Pop. A.R.	1	0.8	0.21		1	4.3	0.96		1	0.30	1.27	
Fst x Paternal Pop. A.R.	1	4.5	1.23		1	0.7	0.15		1	0.19	0.82	
Maternal Pop x Paternal Pop	17	3.6	3.46	***	7	4.5	0.83		21	0.23	0.04	
Residual	471	1.0			166	5.5			716	5.47		

F1 in the field

The F1 in the field from between-population crosses had higher reproductive fitness ($F_{1,7}$=8.5, p<0.001) than plants from within-population crosses and marginally significantly higher vegetative fitness ($F_{1,17}$=4.1, p<0.1). Thus, F1 plants in the field also showed heterosis.

F2 in the greenhouse

F2 fitness after between-populations crosses was not significantly higher than after within-population crosses. However, the interaction competition x cross indicated that F2 vegetative fitness with competition was marginally significantly higher in plants from between than within-population crosses while this was not the case without competition ($F_{1,441}$=2.85, p<0.1; i.e. Figure 4). The effects of between- versus within-population crosses depended on the population for all traits.

Plants from populations with lower allelic richness had a marginally significantly higher vegetative fitness when crosses were performed within populations (cross x maternal allelic richness: $F_{1,7}$=4.64, p<0.1) rather than between populations.

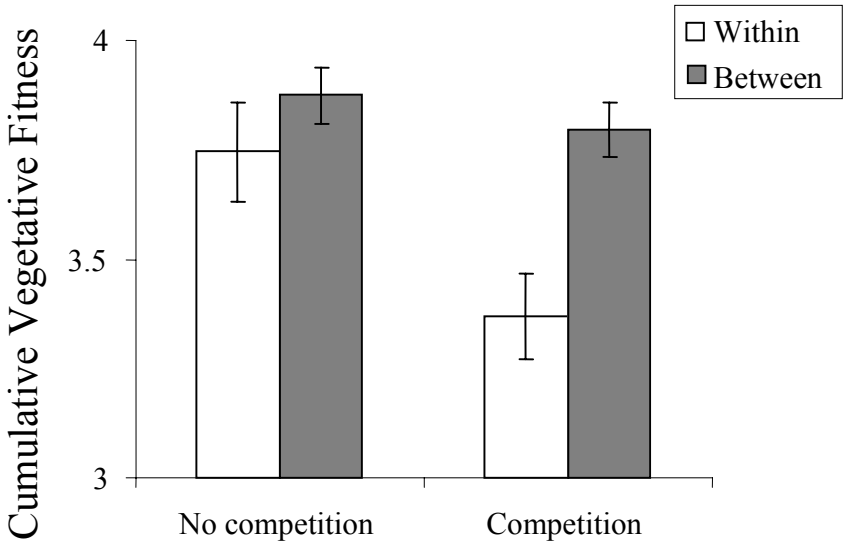

Figure 4: Means of residual vegetative fitness over all plants from each of the 13 natural populations of *Lychnis flos-cuculi* for offspring of F2 plants after within- and between-population crosses without or with competition. F2 vegetative fitness in the greenhouse with competition was marginally significantly higher in plants from between than within population crosses (Competition x Cross interaction, p<0.1). Levels of significance were obtained with hierarchical ANCOVA (Table 4).

Table 4: Analyses of covariance for survival, vegetative and cumulative fitness of F2 in the greenhouse without and with competition after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Effects comprise a set of parental population characteristics variables, and treatment effects. Population denotes the 13 parental populations. We root transformed the data to meet the assumption of normality. We present degree of freedom (df) mean squares (ms) or mean deviance changes (mdev), S = Small, L = Large, Si = Similar, Di = Dissimilar, F and p values : + $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

F2 fitness	**Vegetative**				**Reproductive**				**Survival**			
Source	df	ms	F	p	df	ms	F	p	df	ms	F	p
Maternal Pop. A.R.	1	0.11	0.03		1	2.55	1.05		1	0.14	0.44	
Maternal Pop size	1	2.76	0.72		1	3.98	1.64		1	0.97	2.99	
Maternal Population	9	3.83	1.36		10	2.43	0.53		9	0.32	1.37	
Maternal plants	82	2.81	8.44	***	78	4.55	3.71	***	86	0.24	5.55	***
Competition	1	9.35	58.27	+	1	78.18	232.58	*	1	0.00	0.00	
Cross (W. vs B.)	1	0.40	0.09		1	3.66	1.57		1	0.28	9.76	
Cross Type (S, L, Si, Di)	3	3.30	1.50		3	3.55	0.58		3	0.39	1.66	
Competition x Maternal Pop. A.R.	1	0.16	0.28		1	0.34	0.41		1	0.10	3.29	+
Cross x Maternal Pop. A.R.	1	4.67	4.64	+	1	2.33	1.40		1	0.03	0.22	
Cross Type x Maternal Pop. A.R.	3	2.20	0.62		3	6.09	1.33		3	0.23	1.10	
Competition x Maternal Pop.	10	0.57	1.71	+	10	0.83	0.68		10	0.03	0.74	
Cross x Maternal Pop.	7	1.01	3.02	**	7	1.67	1.36		8	0.13	3.08	**
Cross Type x Maternal Pop.	24	3.53	10.60	***	25	4.57	3.73	***	25	0.21	4.93	***
Competition x Cross	1	0.95	2.85	+	1	0.08	0.06		1	0.01	0.24	
Residual	441	0.33			292	1.23			495	0.04		

Backcrosses

Fitness after backcrosses after between-population crosses was not significantly different from fitness after within-population crosses. Again, the effect of between- versus within-cross and of the four cross types depended on the population for all traits. Plants from populations with lower allelic richness survived marginally significantly better when crosses were performed within populations (cross x maternal allelic richness: $F_{1,6}=5.68$, $p<0.1$; Table 5).

Table 5: Analyses of covariance for survival, vegetative and cumulative fitness of backcrosses in the greenhouse without and with competition after crosses within- and between-populations with plants from 13 natural populations of *Lychnis flos-cuculi*. Effects comprise a set of parental population characteristics variables, and treatments effect. Population denotes the 13 parental populations. We root transformed the data to meet the assumption of normality. We present mean squares (ms) or mean deviance changes (mdev), S = Small, L = Large, Si = Similar, Di = Dissimilar, F and p values: + p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Ba fitness	**Vegetative**				**Reproductive**				**Survival**			
Source	df	ms	F	p	df	ms	F	p	df	ms	F	p
Maternal Pop. A.R.	1	23.2	0.09		1	3.28	0.33		1	0.14	0.70	
Maternal Pop size	1	42.8	0.17		1	0.24	0.02		1	0.02	0.09	
Maternal Population	7	254.4	2.42	*	7	9.87	3.21	**	7	0.20	1.03	
Maternal plants	70	104.9	7.00	***	65	3.07	3.19	***	75	0.19	6.70	***
Competition	1	398.1	63.37	+	1	42.58	144.04	+	1	0.03	18.19	
Cross (W. vs B.)	1	343.5	4.32		1	4.72	0.63		1	0.00	0.00	
Cross Type (S, L, Si, Di)	3	50.1	10.55	*	3	5.89	3.01		3	0.26	1.35	
Competition x Maternal Pop. A.R.	1	6.3	0.75		1	0.30	0.41		1	0.00	0.19	
Cross x Maternal Pop. A.R.	1	79.6	1.46		1	7.48	1.74		1	0.73	5.68	+
Cross Type x Maternal Pop. A.R.	3	4.8	0.05		3	1.96	0.45		3	0.19	1.35	
Competition x Maternal Pop.	8	8.3	0.56		8	0.72	0.75		8	0.01	0.29	
Cross x Maternal Pop.	6	54.5	3.63	**	6	4.29	4.46	***	6	0.13	4.52	***
Cross Type x Maternal Pop.	13	99.7	6.65	***	10	4.34	4.52	***	14	0.14	5.06	***
Competition x Cross	1	4.3	0.29		1	0.00	0.00		1	0.00	0.04	
Residual	285	15.0			197	0.96			311	0.03		

Between-population cross types

For the F1 in the greenhouse, offspring from crosses with larger populations had a lower vegetative fitness compared to the other cross types and offspring from crosses with an ecologically similar population had a higher vegetative fitness (cross type: $F_{1,22}$=3.2, p<0.05).

In contrast, the F1 transplanted to the field did not show any significant differences between the four cross types. Thus, the dichotomy within- versus between-population crosses appeared more important than the specific type of paternal population for offspring fitness under natural conditions.

For the F2, there were no significant main effects of the four cross types. Rather, the effects of the four cross types varied depending on the maternal population.

For backcrosses, the effects of the four cross types varied depending on maternal population and on the considered fitness trait. Crosses with ecologically more different populations produced offspring of lower vegetative fitness compared to the other cross types ($F_{1,3}$=10.6, $p<0.05$).

The effect of the type of cross on backcross performance and both generations of inter-population crosses changed depending on the population. Thus, the effect of fragmentation, as reflected in the genetic diversity of remnant populations did not interact with the type of cross in a consistent way.

Effects of geographic, genetic and ecological distances between populations

For the F1 in the greenhouse the effects of distances between populations were not significant. However, for the F1 transplanted to the field, plant reproductive fitness increased with larger geographical distance between parental populations ($F_{1,7}$=6.1, $p<0.05$) (Figure 5), especially for plants from populations with higher allelic richness ($F_{1,7}$=16.55, $p<0.01$). Plant vegetative fitness increased with absolute difference in humidity indicator values between parental populations ($F_{1,17}$=4.8, $p<0.05$) and plant reproductive fitness increased with absolute difference in acidity indicator values between parental populations ($F_{1,7}$=5.8, $p<0.05$) (Figure 6). To conclude, geographic and ecological distances between populations had an overall positive effect on plant performances after crosses between populations.

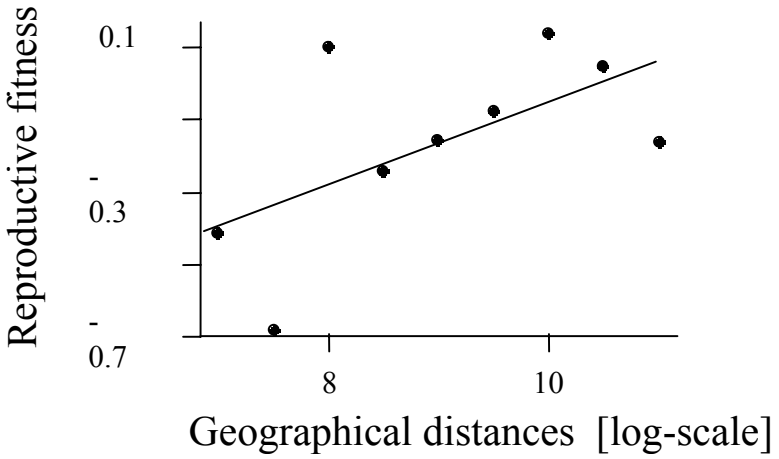

Figure 5: Means of residual reproductive fitness over all plants from each of the 13 natural populations of *Lychnis flos-cuculi* for F1 offspring transplanted to the field after within- and between-population crosses. The positive effect of larger geographic distances for reproductive fitness in the F1 plants transplanted to the field was significant ($p<0.05$). Symbols represent means per population of origin and per transplant target sites, grouped into classes of geographical distances. Levels of significance were obtained with hierarchical ANCOVA (Table 3).

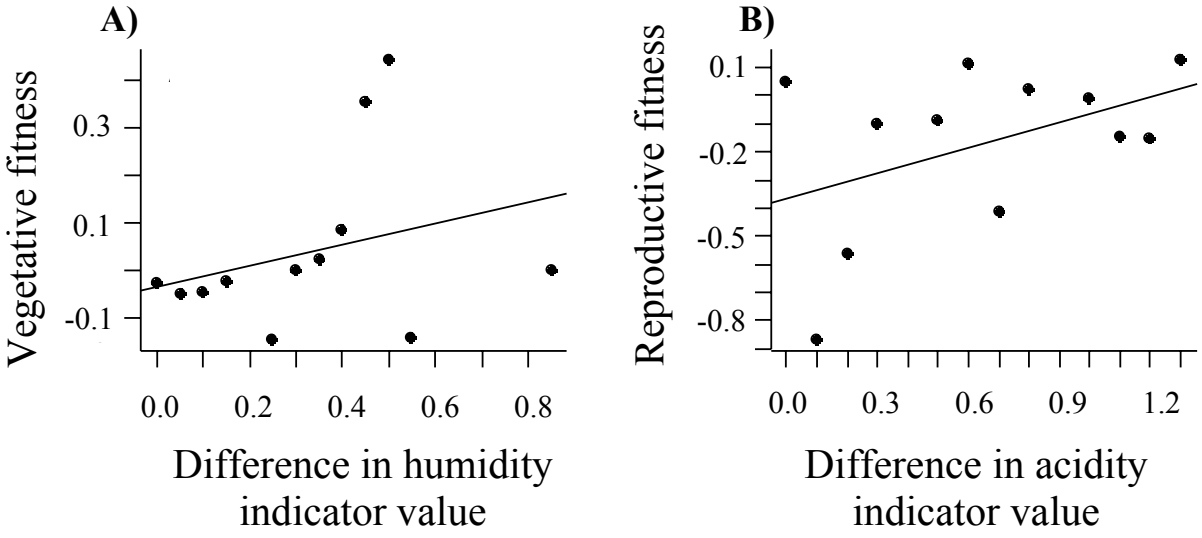

Figure 6: Means of residual fitness over all plants from each of the 13 maternal populations of *Lychnis flos-cuculi* for F1 offspring transplanted to the field after within- and between-population crosses. Effects of ecological distances in humidity were positive for vegetative fitness (A) ($p<0.05$) and in acidity for reproductive fitness (B) ($p<0.05$). Ecological distances were measured as the absolute differences in Landolt (1977) indicator value between the vegetation composition of parental populations. Symbols represent means per population of origin and per transplant target site, grouped into classes of absolute difference of ecological indicator value. Levels of significance were obtained with hierarchical ANCOVA (Table 2).

Discussion

Genetic Allee effect

Offspring of maternal plants from large and genetically more diverse populations performed better than of plants from small populations. This extends earlier results (Galeuchet et al. 2005b) as it concerns offspring resulting from crosses with different populations and as it concerns offspring grown across a range of environmental conditions.

Genetic rescue

Genetic rescue can replenish genetic variation and reduce inbreeding in small populations. Therefore, it usually creates at first a generation of plants which are heterozygous for most loci, and thus deficient alleles are much more likely to be compensated by a normal allele (Frankham 1995). For our 13 populations of *L. flos-cuculi*, both vegetative and reproductive cumulative fitness were higher after between-population crosses in the greenhouse and in the field.

Hauser had found for four populations of *L. flos-cuculi* a higher fitness in the offspring after between-population crosses compared to within-populations crosses (Hauser and Loeschcke 1994), and the same author showed that crosses between populations of *Silene nutans* produced zygotes more likely to survive than zygotes from within-population crosses (Hauser and Siegismund 2000). Similar effects were found for a whole range of species and traits including *Arnica montana* (Luijten *et al.* 2002), *Scorzonera humilis* (Colling *et al.* 2004) and *Silene alba* (Keller *et al.* 2000). The positive heterosis effect in *Silene littorea* was due to reversing inbreeding depression rather than increasing genetic diversity per se (Vilas et al. 2006). Even within populations, Dudash found fitness advantages for far-outcrosses compared to near-outcrosses with *Sabatia angularis*, probably because it reduced the strong inbreeding due to local population structure (Dudash 1990). Thus, heterosis after one generation of outbreeding seems to be the rule rather than the exception. However, the low number of populations involved in

these previous studies did not allow them to test effects of population characteristics or distances between populations on the consequences of between-population crosses.

Outbreeding depression

Most fitness traits in the F1 were non-significantly and vegetative fitness of the F2 and survival of the backcrosses were marginally significantly higher for within-population crosses for populations with low allelic richness. This suggests tendencies for some outbreeding depression for plants of small, genetically depauperate populations. This could be due to purging of deleterious alleles in smaller populations. Indeed the fitness of between-populations crosses varied little across different population sizes, whereas the fitness of within-population crosses decreased significantly with maternal population size. On the other hand, it could be explained by the action of genetic drift on plant genomes. Mixing different gene pools could then break-up co-adapted gene complexes.

In an experiment with *Papaver rhoeas,* biomass was negatively influenced by outbreeding caused by epistasis in all four generations, and seed mass was decreased in the F2 (Keller *et al.* 2000). Outbreeding depression was observed in *Gentianella germanica* after crosses between populations (Fischer and Matthies 1997). Waser and co-authors found that outbreeding effects vary for the same species in space and time (Waser *et al.* 2000). These examples illustrate why gene flow has been termed the Jekyll and Hyde of conservation (Stockwell *et al.* 2003).

No break-up of local adaptation

Plants transplanted to the field populations did not show a break-up of local adaptation, although a reciprocal replant-transplant experiment reported that *L. flos-cuculi* plants from our populations are adapted to ecological gradients (Bowman et al., in prep). Moreover, in the field plants showed a similar heterosis effect than in the greenhouse. A similar advantage for plants from between-populations crosses compared to within-population crosses was found for *Scabiosa columbaria* between populations at distances

of up to 160 km (Van Treuren *et al.* 1993), which corresponds to the geographical range of our study.

However, other studies found a break-down of local adaptation, even at a small scale. Within the same population of *Impatiens capensis*, outbred plants were doing worse than the inbred plants when growing exactly on the same site than the maternal plants, while the opposite was true for offspring growing further away from the maternal plant (Schmitt and Gamble 1990). Although the importance of choosing the right source for introduction of plant material was stressed in conservation and the need declared to delineate seed transfer zones (Hufford and Mazer 2003) our experiment suggests that this might not always be important. Rather, adequate seed transfer zones might be quite large.

Effects of geographic, genetic and ecological distances between populations

Offspring fitness increased monotonically with increasing geographic and ecological distances between populations. Thus, we did not detect an optimum outbreeding distance (Waser 1993a, Willi and Van Buskirk 2005). Possibly, at even further crossing distances than the ones used in our study, maximum offspring fitness might have been reached.

This confirms that the break-up of local adaptation does not occur in L*ychnis flos-cuculi* within this range of geographic and ecological distances. Thus, when introducing plant material from distant sources break-up of local adaptation might be less important than previously thought.

Conclusion

Our study with many populations allowed us to evaluate the effects of population characteristics, and of several types of inter-population distances for consequences of inter-population crosses. Overall, we conclude that heterosis outweighs potentially negative effects of inter-population crosses, at least in *Lychnis flos-cuculi*.

Acknowledgments

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Chapter 6

Summary

Zusammenfassung

Résumé

Summary

In the last century, and especially in the last fifty years, changes in agricultural land use, and development and urbanization, have thoroughly transformed the landscapes of Central Europe. The main consequences of modern development for plants are habitat changes, which made many formerly available habitats unsuitable for many species, and habitat fragmentation, which reduces the size and increases the distance between remaining habitats. Consequently, many natural populations even of common plant species have become small and isolated.

For a number of reasons such populations have been predicted to be at higher risk of extinction than larger and less isolated populations. They are more vulnerable to demographic and environmental stochasticity. Moreover, genetic drift and inbreeding are more important in small and isolated populations, reducing genetic variation, and, because of inbreeding depression, also plant fitness. Allee effects, defined as negative effects of low density or size of populations on individual fitness, can also come about by increased prevalence or impact of biotic interactions of plants with herbivores and pathogens in smaller and more isolated populations.

Previous studies of demographic and genetic consequences of landscape fragmentation for plants mainly focused on rare plant species. Moreover, previous studies either covered short-lived plants, only single field seasons, only so few populations, that no conclusions on population size effects could be drawn, or they used single-generation common garden studies.

Therefore, I studied effects of fragmentation on a common plant species, the ragged robin *Lychnis flos-cuculi* (Cariophyllaceae), which is common in wet grasslands in Europe. In Switzerland, where I studied the species, 90% of wetlands disappeared in the last century. Therefore, wet natural habitats have become scarce, small, and isolated. This situation makes *L. flos-cuculi* in wet grasslands in Switzerland a good model to investigate effects of landscape fragmentation on a long-lived perennial. I investigated plant performance in many field populations for five years, monitored population sizes and used matrix models to investigate the consequences of variation in plant performance for population performance. Moreover, I used three generations of experimental

inbreeding and within-population outcrossing for plants from many populations to assess consequences of longer-term inbreeding for populations of different size and isolation. Finally, I studied consequences of two generations of between-population crosses for offspring fitness to evaluate the potential of artificial gene flow between populations as a measure for conservation and restoration. Such artificial gene flow may be beneficial because it counteracts the loss of genetic variability in smaller and more isolated populations. However, heterosis effects may be limited to the first generation, while offspring fitness may be decreased in the second generation following between-population crosses because of a break-down of local adaptation or of co-adapted gene complexes.

In the first results chapter of this thesis, **Chapter 2**, I report on individual fitness of plants of *L. flos-cuculi* in 26 natural populations in relation to environmental conditions and habitat fragmentation. In each population, the performance of 24 to 48 plants was monitored for 5 years. At higher altitudes, rosettes had fewer leaves, and flowering plants had fewer flowers. Moreover, plants produced fewer rosette leaves in populations with lower light indicators. Plant damage by herbivores was less likely in populations at higher altitudes. In the extremely dry year 2003 pathogen occurrence increased while the occurrence of herbivory decreased. Lower rates of plant survival in smaller populations indicate an Allee effect. Rates of plant survival were also lower in the more isolated of our studied populations. Reproductive plants were more likely to be affected by fungal pathogens in smaller populations. With few exceptions, effects of population characteristics on plant performance did not differ between study years. I conclude that plant performance in different field populations of *Lychnis flos-cuculi* in Switzerland is not only affected by environmental differences, but to a large degree, via high degrees of population isolation and small population sizes, also by landscape fragmentation.

Chapter 3 reports significant variation in population size of *Lychnis flos-cuculi*. I counted population sizes at the peak of flowering in June of 2000, 2001, 2003, and 2004 and I showed that populations are sensitive to environmental conditions and particularly to drought. Moreover, with matrix analyses elaborated from the observation of 24 to 48 plants in each population, I calculated finite growth rates of populations (λ) which were between 1 and 1.04 for all populations, indicating quite stable growth of populations. Moreover, λ of smaller populations tended to be smaller than for larger ones. However, observed proportion of plants in the different stages of the life cycle deviated from the projected stable stage vector, indicating that the studied populations are not in equilibrium. In smaller and more isolated populations, the proportion of vegetative plants was especially high. Stochastic simulations of finite population growth rates and extinction rates over 50 years, based on 1000 runs revealed lower average population growth rates and higher extinction rates of smaller and more isolated populations. Our results indicate that all our study populations are facing the risk of extinction within the next 100 years, and especially so the smaller and more isolated ones.

In the fragmented landscape, isolation of populations and small population size increase rates of inbreeding. This may lower plant fitness via inbreeding depression. At the same time, inbreeding depression may have been purged from smaller and more inbred populations, or may be purged more rapidly from such populations if a regime of inbreeding sets in. **Chapter 4** tests the consequences of experimental inbreeding for *Lychnis flos-cuculi* plants from 19 populations of different size and isolation. Three generations of crosses of different degrees of experimental selfing and outcrossing (within population) were performed. Inbreeding depression was expressed for all generations and at the stages of seed production, germination, and adult plant size. Plant fitness decreased significantly with increasing experimental inbreeding coefficient, showing evidence of inbreeding depression in more inbred lines compared to outbred lines. However, depending on the history of populations, there were also indications of purging of inbreeding load. After three generations of inbreeding, inbreeding depression tended to be reduced in less genetically diverse populations. Overall, this chapter suggests that common plants such as *Lychnis flos-cuculi* suffer from inbreeding

depression and do not purge rapidly their inbreeding load. Its populations are therefore prone to negative genetic consequences of landscape fragmentation.

Given the negative consequences of inbreeding within populations, **Chapter 5** evaluates whether between-population crosses are beneficial for the fitness of *Lychnis flos-cuculi* plants. I crossed plants of each of 13 populations population with a plant from the same population, with a plant from a small, from a large, from an ecologically similar and from an ecologically dissimilar population. Offspring grew until flowering both in the greenhouse and in the field populations of origin. Moreover, I used the greenhouse plants to produce F2 and backcrosses. Offspring of crosses between populations had higher vegetative and reproductive cumulative fitness in the F1 both in the greenhouse and in the field, indicating heterosis rather than breaking-up of local adaptation. Although the heterosis effect was still positive in the F2 and backcrosses, it was not significant any more. Nevertheless, overall our study suggests that heterosis after between population crosses outweighs potential negative consequences.

Conclusion

My thesis adds several new insights to the study of effects of landscape fragmentation for plants. It shows that negative effects of fragmentation on individual plant fitness are largely consistent across five study years, and that these effects are not masked by environmental differences between populations. Moreover, it shows that these effects on individual fitness lead to decreased population growth of smaller and more isolated populations. Moreover, stochastic simulations based on the field data predict higher risks of extinction for smaller and more isolated populations. The three-generation inbreeding experiment demonstrates that considerable inbreeding depression is consistent among populations, implying that it has not been purged from smaller populations. Moreover, the three generations of experimental selfing showed that inbreeding depression is also not likely to be purged in the future.

These findings are even more substantial, as negative consequences of fragmentation are assumed to become apparent later in long-lived perennials than in shorter-lived plants. Therefore, it is very well possible that the observed negative effects

of fragmentation are only the beginning, and that the future development of *Lychnis flos-cuculi* populations is even more affected by fragmentation. Moreover, the recent climatic changes, especially warming, observed in our regions could further accelerate the decline of this wetland species by changing its environment. Moreover, reduced genetic variation in small and isolated populations reduces the potential of such populations to adapt to changed environmental conditions.

Several measures have been suggested to prevent the negative effects of fragmentation for plants and my results support the importance of such measures. A further loss of wetlands has to be prevented and natural habitats have to be protected as first priority and any loss of such habitats has to be compensated adequately. Moreover, natural habitats have to be maintained at a size large enough to prevent strong genetic drift and inbreeding. Finally, wet habitats should be connected in habitat networks to allow sufficient gene flow via pollinator movement or seed dispersal to maintain genetic diversity of populations. Finally, restoration measures should consider enhancing genetic variability of small and inbred populations by artificial gene flow.

Zusammenfassung

Im letzten Jahrhundert und speziell in den letzten 50 Jahren haben die landwirtschaftliche Landnutzung, der Fortschritt allgemein sowie die Verstädterung das Landschaftsbild in Zentraleuropa einschneidend verändert. Die Hauptkonsequenzen dieser modernen Entwicklung sind einerseits die für viele Arten ungünstigen Veränderungen in den Habitaten selbst und andererseits die Fragmentierung der Landschaft. Letzteres führt dazu, dass die Habitate immer kleiner und die Distanzen dazwischen immer grösser werden. Natürliche Populationen, auch diejenigen häufig vorkommender Pflanzenarten, werden dadurch immer kleiner und isolierter.

Aus verschiedensten Gründen sind kleine und isolierte Populationen einem grösseren Aussterberisiko ausgesetzt als grössere und weniger isolierte Populationen. Sie sind anfälliger für zufällige demographische und umweltbedingte Schwankungen. Inzucht und genetische Drift wirken sich stärker aus. Zudem kommt es aufgrund der Inzuchtdepression zu einer Verminderung der Fitness. Nimmt die Häufigkeit der biotischen Interaktionen zwischen Pflanze und Pflanzenfresser bzw. Krankheitserreger zu, unterliegen die kleinen und isolierten Populationen ausserdem einem Allee-Effekt. Allee-Effekte sind definiert als negative Auswirkungen auf die Fitness von Individuen bei verringerter Populationsdichte oder -grösse.

Frühere Studien über die demographischen und genetischen Konsequenzen der Landschaftsfragmentierung auf Pflanzen konzentrierten sich vor allem auf seltene Arten. Dazu kommt, dass die meisten dieser Studien nur kurzlebige Pflanzen untersuchen, sich auf eine Feldsaison oder auf eine kleine Anzahl Populationen beschränken. Vergleichende Studien mit zu wenigen Populationen erlauben jedoch keine Schlüsse über die Auswirkungen der Populationsgrösse.

Deshalb untersuchte ich die Auswirkungen der Fragmentierung an einer häufig vorkommenden Pflanzenart, der Kuckuckslichtnelke *Lychnis flos-cuculi* (Cariophyllaceae), die in Europa vor allem in Feuchtwiesen wächst. In der Schweiz sind im letzten Jahrhundert rund 90 % der Feuchtgebiete verschwunden. Folglich sind auch die Feuchtwiesen seltener, kleiner und isolierter geworden. *Lychnis flos-cuculi* ist deshalb ein ideales Untersuchungsobjekt, um die Auswirkungen der Landschaftsfragmentierung

an einer mehrjährigen Pflanze zu untersuchen. Während fünf Jahren untersuchte ich in Flachmooren in den Schweizer Kantonen Appenzell, St. Gallen und Schwyz in zahlreichen Populationen Fitnessmerkmale markierter *Lychnis*- Pflanzen. Mit Hilfe von mehrjährigen Zählungen der Populationsgrössen und Matrizenmodellen der Populationsentwicklungen untersuchte ich die Konsequenzen der unterschiedlichen Fitnessmerkmale der einzelnen Pflanzen auf die Fitness der Populationen. Ausserdem verwendete ich drei Generationen aus experimenteller Selbstbestäubung und Kreuzungen zwischen Pflanzen innerhalb der Populationen, um die Konsequenzen von längerfristiger Inzucht auf Populationen unterschiedlicher Grösse und unterschiedlichen Isolationsgrades zu dokumentieren. Schlussendlich untersuchte ich auch die Fitnesskonsequenzen von zwei Generationen von Kreuzungen zwischen Paaren von Populationen auf die Fitness der Nachkommen, um damit das Potential des künstlichen Genflusses zwischen Populationen als Massnahme für die Erhaltung und das Management von kleinen bedrohten Populationen zu evaluieren. Ein solcher künstlicher Genfluss könnte durchaus nützlich sein, weil er in kleinen und isolierten Populationen dem Verlust der genetischen Variabilität entgegenwirken kann. Allerdings beschränkt sich der so genannte Heterosiseffekt, also die erhöhte Fitness nach Kreuzung zwischen Populationen, oft auf die erste Nachkommengeneration, während die Fitness der Nachkommen der zweiten Generation – aufgrund des Zusammenbruchs der lokalen Anpassung oder der gemeinsamen Anpassung von Genkomplexen – abnimmt.

Im ersten Kapitel der Resultate, **Kapitel 2**, berichte ich über die individuelle Fitness von Pflanzen in 26 natürlichen *Lychnis flos-cuculi*-Populationen mit Hauptaugenmerk auf deren Zusammenhang mit Landschaftsfragmentierung und Umweltbedingungen. Während fünf Jahren wurden für jede Population 24 bis 48 Pflanzen auf ihre Fitnessmerkmale untersucht. In höheren Lagen fanden wir Rosetten mit weniger Blättern und weniger Blüten bei blühenden Pflanzen. In Populationen mit geringerer Lichteinstrahlung anzeigender Vegetation wurden weniger Rosettenblätter produziert. Die Wahrscheinlichkeit, durch Herbivorie Schaden zu erleiden, war in höheren Lagen kleiner. Im Hitzesommer 2003 nahm der Befall von Pathogeninfektionen zu, während der Schaden durch Herbivorie abnahm. Die tiefere Überlebensrate in

kleineren Populationen deutet auf einen Allee-Effekt hin. Auch die Pflanzen der isolierten Populationen im Untersuchunggebiet zeigen eine tiefere Überlebensrate. In kleineren Populationen wurden die reproduktiven Pflanzen vermehrt von Pilzen befallen. Mit wenigen Ausnahmen unterschieden sich diese Auswirkungen der Populationseigenschaften auf die Pflanzenfitness nicht zwischen den verschiedenen Untersuchungsjahren. Wir schlussfolgern, dass die Fitnessmerkmale von *Lychnis flos-cuculi* in den unterschiedlichsten Feldpopulationen in der Schweiz nicht nur von Umweltbedingungen beeinflusst werden, sondern hauptsächlich auch von der Landschaftsfragmentierung, was sich deutlich im hohen Grad der Isolation von Populationen und deren Grösse manifestiert.

Das **Kapitel 3** befasst sich mit der Variation der Populationsgrössen und der Populationswachstumsraten von *Lychnis flos-cuculi*. Wir untersuchten die Populationsgrösse während der Blütezeit im Juni in den Jahren 2000, 2001, 2003 und 2004. Wir konnten dabei zeigen, dass die Populationsgrösse empfindlich auf Umwelteinflüsse, speziell auf Trockenheit, reagierte. Mit Hilfe der Projektionsmatrizenanalyse, resultierend aus Messungen an 24 bis 48 Pflanzen pro Population, berechneten wir die Wachstumsrate (λ) der Populationen. Die Berechnungen ergaben für alle Populationen ein λ zwischen 1 und 1.04. Diese Werte deuten auf ein stabiles Wachstum der untersuchten Populationen hin. Allerdings konnten wir zeigen, dass das λ von kleinen Populationen kleiner ausfällt, als dasjenige von grösseren Populationen. Aufgrund eines Überwiegens von vegetativen Pflanzen weicht die Populationsstruktur der beobachteten Proportionen von Pflanzen in den verschiedenen Wachstumsstadien von der durch die Eigenvektoren der Projektionsmatrizen gegebenen Vorhersage der stabilen Populationsstruktur ab, was darauf hindeutet, dass sich die untersuchten Populationen nicht in einem Gleichgewicht befinden. Mit Hilfe von stochastischen Projektionssimulationen (1000 Wiederholungen) errechneten wir die Wachstums- und Aussterberate der Populationen für die nächsten 50 Jahre. Die Berechnungen zeigten für kleine und isolierte Populationen eine durchschnittlich tiefere Wachstumsrate sowie eine höhere Aussterberate. Dieses Resultat verdeutlicht, dass

sämtliche von uns untersuchten Populationen in den nächsten 100 Jahren vom Aussterben bedroht sind, vor allem kleinen und isolierten Populationen.

In einer fragmentierten Landschaft nimmt die Inzuchtrate bei kleinen und isolierten Populationen zu. Dies könnte via Inzuchtdepression zu einer Reduktion der Fitness führen. Allerdings könnte die Inzuchtdepression bei dort höhererer Inzucht vor allem aus kleineren und isolierteren Populationen ganz eliminiert oder zumindest schneller eliminiert werden. **Kapitel 4** befasst sich deshalb mit den Konsequenzen experimenteller Inzucht für Pflanzen von 19 *Lychnis flos-cuculi*-Populationen verschiedener Grössen und Isolationsgrade. Wir haben dazu Kreuzungen für drei Generationen mit unterschiedlicher experimenteller Selbstbestäubung sowie Kreuzungen innerhalb von Populationen durchgeführt. Inzuchtdepression fanden wir in allen drei Generationen, sowohl im Stadium der Samenproduktion und der Keimung, als auch in der adulten Pflanze. Bei Zunahme des Inzuchtkoeffizienten nahm dabei die Fitness ab, was Inzuchtdepression anzeigt. Abhängig von der genetischen Diversität einer Population gab es auch Hinweise für ein Ausmerzen der Inzuchtlast durch Selektion gegen mehr ingezüchtete Pflanzen. Nach drei Inzuchtgenerationen verringerte sich nämlich die Inzuchtdepression in Populationen mit geringerer genetischer Diversität. Zusammengefasst zeigen die Resultate dieses Kapitels, dass häufig vorkommende Pflanzenarten wie *Lychnis flos-cuculi* unter Inzuchtdepression leiden und dass sie ihre Inzuchtlast nicht schnell durch das oben erwähnte Ausmerzen reduzieren. Die Landschaftsfragmentierung hat deshalb auf ihre Populationen negative genetische Auswirkungen.

Nachdem wir herausgefunden hatten, dass Inzucht negative Fitnessauswirkungen innerhalb von Populationen nach sich zieht, untersuchten wir in **Kapitel 5**, ob sich Kreuzungen zwischen Populationen positiv auf die Fitness von *Lychnis flos-cuculi* auswirken. Dazu kreuzten wir Pflanzen aus 13 Populationen mit Pflanzen aus der eigenen, aus einer kleinen, grossen, ökologisch ähnlichen und ökologisch unterschiedlichen Population. Die Kreuzungsversuche fanden im Treibhaus statt und Nachkommen wurden sowohl im Treibhaus als auch im Feld in den Originalpopulationen

aufgezogen. Im Treibhaus wurden auch F2-Generationen und Rückkreuzungen erzeugt. Die Nachkommen der Kreuzungen zwischen Populationen zeigten eine höhere Gesamtfitness der F1-Generation als der Elterngeneration, sowohl im Treibhaus als auch im Feld. Dies deutet eher auf Heterosis als auf einen Zusammenbruch der lokalen Anpassung hin. Dieser Heterosiseffekt blieb auch in der F2-Generation und den Rückkreuzungen weiterhin positiv, wenn auch nicht mehr statistisch signifikant. Insgesamt deutet unsere Studie darauf hin, dass die Heterosis bei Kreuzungen zwischen Populationen die potentiell negativen Konsequenzen überwiegt.

Schlussfolgerungen

Meine Dissertation beschreibt neue Erkenntnisse über die Auswirkungen der Landschaftsfragmentierung auf Pflanzen. Sie zeigt deutlich, dass sich die Landschaftsfragmentierung negativ auf die Fitness der einzelnen Pflanzen auswirkt. Dies gilt für sämtliche fünf Untersuchungsjahre und wird auch nicht durch die unterschiedlichen Umweltbedingungen, denen die Populationen ausgesetzt sind, verwischt. Die Auswirkungen auf die Pflanzenfitness führen schliesslich zu einer Verminderung der Wachstumsrate bei kleineren und isolierteren Populationen. Ausserdem verdeutlichen auf den Felddaten basierende stochastische Simulationen der Populationsentwicklungen die Probleme kleinerer und isolierterer Populationen, für die die Simulationen ein grösseres Aussterberisiko ergeben. Anhand der Inzuchtversuche über drei Generationen konnten wir demonstrieren, dass die Inzuchtdepression für alle Populationen gross und konsistent ist und auch in den kleinen Populationen nicht verschwindet. Weiter zeigen die experimentellen Selbstbestäubungsversuche, dass die Inzuchtdepression auch in Zukunft nicht eliminiert werden kann.

Diese Resultate sind umso bedeutender, als sich die negativen Effekte der Landschaftsfragmentierung in mehrjährigen Pflanzen erst später manifestieren als in kurzlebigen Pflanzen. Wir können daher annehmen, dass es sich bei den beobachteten negativen Auswirkungen erst um einen Anfang handelt, und dass die Entwicklung von *Lychnis flos-cuculi* in Zukunft noch stärker durch die Fragmentierung betroffen sein wird. Weiter werden auch die in unseren Breitengraden beobachteten Klimaveränderungen und speziell die Erderwärmung den Rückgang von Pflanzenarten in den Feuchtgebieten

beschleunigen. Schliesslich verringert die verminderte genetische Variabilität in den kleinen und isolierten Populationen die Chance, dass sich diese Populationen an die neuen Umweltbedingungen anpassen können.

Verschiedene Massnahmen können die negativen Auswirkungen der Landschaftsfragmentierung auf die Pflanzen abschwächen. Meine Arbeit untermauert die Wichtigkeit solcher Massnahmen. An erster Stelle muss verhindert werden, dass weitere Feuchtgebiete verloren gehen. Die verbleibenden natürlichen Habitate müssen also unbedingt geschützt werden. Der Verlust von Habitaten muss adäquat kompensiert werden. Weiter müssen natürliche Habitate eine entsprechende Grösse aufweisen, damit eine starke genetische Drift und Inzucht verhindert werden können. Schliesslich sollten Feuchtgebiete in eine vernetzte Landschaft eingebettet sein, damit ein ausreichender Genfluss via Pollen- oder Samenverbreitung gewährleistet wird und damit ausreichende genetische Diversität in den Populationen aufrechterhalten bleibt. Nicht zuletzt sollten auch Massnahmen des künstlichen Genflusses in Betracht gezogen werden, die die genetische Variabilität in den kleinen und ingezüchteten Populationen erhöht.

Résumé

L'important développement de l'agriculture et de l'urbanisation a, depuis un siècle, complètement modifié les paysages d'Europe centrale. Et les conséquences principales de cette modernisation sont plus particulièrement observables depuis 50 ans, au niveau des habitats naturels. Ceux-ci deviennent inadéquats et ne permettent plus aux populations naturelles, en particulier aux populations végétales, d'y vivre. D'autre part, les habitats sont de plus en plus fragmentés ce qui engendre une réduction de la taille des populations et une perte de connectivité entre elles. Par conséquent, un certain nombre de populations naturelles sont devenues petites et isolées, y compris pour des plantes communes.

Les populations qui sont réduites en taille et isolées présentent, pour plusieurs raisons, un taux d'extinction plus élevé que les grandes populations ayant une bonne connectivité entre elles. En premier lieu, les petites populations montrent une sensibilité plus marquée face à la stochasticité démographique et environnementale à laquelle elles sont soumises. Ensuite, la diversité génétique est réduite dans ces populations par l'action de la dérive génétique et de l'apparition de consanguinité qui, par la dépression de consanguinité, réduit la fitness des plantes. De plus, les effets négatifs liés à une réduction en taille ou en densité des populations, nommés effets Allee, peuvent s'ajouter et augmenter l'impact des interactions biotiques entre plantes et herbivores, ou pathogènes, sur la fitness des plantes dans les petites populations.

Les recherches précédentes concernant les effets démographiques et génétiques de la fragmentation du paysage se sont principalement focalisées sur des espèces végétales rares. D'autre part, ces recherches étaient souvent axées sur des plantes annuelles, ou de courte durée de vie. Enfin, trop fréquemment, les études se sont déroulées sur une seule et unique saison et/ou le nombre de populations étudiées était très petit. Ainsi, aucune conclusion concernant les effets de la taille des populations n'a pu être établie.

Afin de remédier à ces quelques lacunes, ce travail de doctorat s'est concentré sur l'étude des effets de la fragmentation du paysage pour une plante décrite comme commune, la Silene fleur de coucou (*Lychnis flos-cuculi, (Cariophyllaceae)*). Cette plante est généralement abondante dans les prairies humides de Suisse et d'Europe. Cependant, en Suisse, 90% des zones humides ont disparues depuis 100 ans et les habitats naturels

humides sont devenus rares, petits et isolés. La situation actuelle de cette espèce (*L. flos-cuculi*), la diminution de son habitat et son statut de plante vivace en fait un très bon modèle pour étudier les effets de la fragmentation du paysage en Suisse. J'ai étudié la performance des plantes dans plusieurs populations durant 5 ans, j'ai suivi l'évolution de la taille des populations et utilisé un model matriciel pour mesurer les conséquences d'une variation dans la fitness des plantes sur la performance des populations elles-mêmes. De plus, j'ai effectué pour un nombre important de populations, qui diffèrent en taille et en isolation, trois générations de croisements artificiels afin d'augmenter la consanguinité et d'en mesurer les conséquences à long terme. J'ai également effectué, parallèlement, les croisements complémentaires entre plantes au sein des populations afin d'obtenir une comparaison avec les lignes consanguines. Finalement, j'ai effectué deux générations de croisements entre populations, et j'ai mesuré la fitness des descendants afin d'estimer les effets des croisements inter-populations. J'ai donc évalué les effets d'une augmentation artificielle du flux de gènes entre populations dans le but d'estimer l'efficacité des mesures de conservation et de restauration des milieux naturels. Une augmentation artificielle du flux de gènes peut être bénéfique pour les populations car elle contrebalance la perte en variabilité génétique observable dans les petites populations et les populations isolées. Cependant, cet effet positif, appelé 'heterosis', peut diminuer à la seconde génération de croisements déjà, car des croisements entre populations, ayant des conditions environnementales différentes, peuvent détruire soit l'adaptation locale des plantes, soit des complexes de gènes co-adaptés présents dans leur génome.

Dans le premier chapitre de mon doctorat, **Chapitre 2**, j'ai étudié la fitness des plantes de 26 populations naturelles de *Lychnis flos-cuculi,* en relation avec les conditions environnementales et la fragmentation du paysage. Dans chaque population, j'ai mesuré la performance de 24 à 48 plantes durant 5 ans. Les plantes situées à des altitudes plus élevées présentent des rosettes avec moins de feuilles et des plantes reproductrices avec moins de fleurs. Les plantes végétatives des habitats moins lumineux présentent un nombre réduit de feuilles. De plus, les populations d'altitude présentent également un nombre restreint d'attaques d'herbivores. J'ai également observé durant l'été 2003, qui fut particulièrement chaud et sec, que la présence des pathogènes a augmenté alors que

celle des herbivores a diminué. Concernant les effets directs de la fragmentation, la présence d'effets Allee est clairement indiquée par les taux de survie réduits mesurés dans les petites populations. Parallèlement, les taux de survie des populations isolées est également significativement plus bas et, finalement, les plantes reproductives sont plus touchées par les pathogènes dans les petites populations. Pour confirmer la robustesse des ces résultats, il est important de signaler que, sauf pour quelques rares exceptions, les effets engendrés par les caractéristiques des populations ne diffèrent pas entre les années. On peut donc conclure que la performance des plantes, dans les différentes populations naturelles de *Lychnis flos-cuculi* en Suisse, n'est pas seulement affectée par les conditions environnementales, mais aussi, via un degré élevé d'isolation et de populations réduites en taille, par la fragmentation du paysage.

Le **Chapitre 3** met tout d'abord en évidence une variation significative de la taille des populations de *Lychnis flos-cuculi*. Les populations ont été comptées au moment maximum de la floraison, c'est-à-dire en juin 2000, 2001, 2003 et 2004. Par ce suivi, j'ai pu démontrer que les populations sont sensibles aux conditions environnementales et en particulier à la sécheresse. Ensuite, à l'aide d'une analyse matricielle, élaborée à partir de l'observation de 24 à 48 plantes dans chaque population, j'ai pu calculer les taux de croissance (λ) des populations. Ces taux de croissance se situent entre 1 et 1.04 pour toutes les populations, indiquant une croissance stable des populations. Cependant, les taux de croissance des petites populations tendent à être plus bas comparés à ceux des grandes populations, cela suggère que les petites populations sont plus faibles face aux événements stochastiques. De plus, même si les populations montrent un taux de croissance proche de la stabilité, la comparaison entre les vecteurs d'âge stable calculés, et ceux observés sur le terrain, indique que les populations ne présentent pas une structure stable au niveau des stades de croissance, traduisant d'une perturbation de la démographie des populations. Finalement, j'ai estimé par simulation stochastique les taux de croissance (λ) et les taux d'extinction des populations sur une période de 50 ans et sur un total de 1000 simulations. De ces simulations il apparaît que les taux de croissance des petites populations isolées sont en moyenne plus bas que les taux de croissance des autres populations. De façon similaire, le taux d'extinction, estimé sur 1000 simulations, est en

moyenne plus élevé pour les petites populations isolées. Ces résultats suggèrent clairement que les populations étudiées font face à un risque d'extinction important, en particulier celles qui sont petites ou isolées.

Dans un contexte de fragmentation du paysage, l'isolation et la petite taille des populations engendrent une augmentation du taux de consanguinité qui, par la dépression de consanguinité, réduit la fitness des plantes. Simultanément, la dépression de consanguinité peut être éliminée (purge génétique) dans les petites populations ou les populations les plus isolées. Le **Chapitre 4** teste les conséquences, à long terme, de la consanguinité pour *Lychnis flos-cuculi*. J'ai expérimentalement croisé, durant trois générations, les plantes de 19 populations qui diffèrent en taille et en isolation afin d'obtenir différents degrés de consanguinité. Parallèlement, j'ai effectué des croisements contrôles entre plantes différentes, mais toujours provenant de la même population. Les résultats montrent clairement la présence de dépression de consanguinité dans toutes les générations et à tous les stades de développement des plantes, allant de la production des graines et de la germination à la taille adulte. En effet, la fitness des plantes est significativement réduite lorsque le taux de consanguinité expérimental augmente, mettant en évidence une dépression de consanguinité dans les lignes consanguines par rapport aux lignes de croisements contrôles. Cependant, en fonction de l'histoire des populations, j'ai pu observer quelques indications suggérant la présence de purge génétique. C'est seulement après trois générations de croisements consanguins que la dépression de consanguinité tend à être réduite dans les populations génétiquement les plus pauvres. De manière plus générale, ce chapitre suggère qu'une plante comme *Lychnis flos-cuculi*, qui est décrite comme commune, souffre de la dépression de consanguinité et n'élimine pas rapidement le fardeau génétique lié à cette consanguinité. Les populations sont donc sujettes aux conséquences génétiques négatives liées à la fragmentation du paysage.

Étant donné les conséquences négatives évidentes de la consanguinité au sein des populations, le **Chapitre 5** évalue le bénéfice de croisements entre populations pour la fitness de *Lychnis flos-cuculi*. Des croisements entre plantes provenant de 13 populations

différentes ont été effectués et 5 types de croisements différents ont été appliqués ; un croisement avec une plante provenant de la même population, avec une plante provenant d'une petite population, avec une plante provenant d'une grande population, avec une plante provenant d'une population similaire d'un point de vue écologique et d'une plante provenant d'une population dissimilaire d'un point de vue écologique. Les descendants de ces croisements ont grandit en serre et sur le terrain jusqu'à la floraison. De plus, les croisements retour avec les plantes mères ont été effectués en serre pour la seconde génération. Les descendants issus des croisements entre populations présentent une meilleure fitness en serre et sur le terrain, ce qui indique un effet 'heterosis' plutôt qu'une destruction de l'adaptation locale. Bien que l'effet heterosis fut encore présent à la seconde génération, mais plus de manière significative, cette étude suggère que l'effet 'heterosis' contre balance les effets négatifs potentiels de croisements entre population.

Conclusion

Mon travail de doctorat apporte de nouvelles connaissances concernant les effets de la fragmentation du paysage sur les espèces végétales. Il montre que les effets négatifs de cette fragmentation sur la fitness individuelle des plantes sont constants au travers de cinq années d'étude et que ces effets ne sont pas masqués par les différences environnementales entre populations. De plus, ce travail démontre que les effets observés mènent à une réduction de croissance pour les petites populations et les populations isolées. Les simulations stochastiques, basées sur les données récoltées sur le terrain, s'ajoutent à ces résultats pour prédire un risque d'extinction particulièrement élevé pour les petites populations et les populations isolées. L'expérience concernant les trois générations de consanguinité démontre clairement que la dépression de consanguinité est considérable et constante entre les différentes populations, indiquant que le purging n'apparaît pas rapidement dans les petites populations. Après trois générations de consanguinité il apparaît seulement une tendance au purging, laissant supposer que l'élimination future du fardeau génétique, engendrant la dépression de consanguinité, ne se mettra pas en place rapidement.

Ces découvertes sont d'autant plus substantielles que les conséquences négatives de la fragmentation du paysage sont supposées apparaître plus tard chez les plantes

vivaces par rapport aux plantes ayant une durée de vie plus courte. Ainsi, il est hautement probable que les effets observés ici ne sont que le commencement d'autres effets plus importants. De ce fait, le développement futur des populations de *Lychnis flos-cuculi* sera de plus en plus affecté par la fragmentation. De plus, les changements climatiques récents, plus particulièrement le réchauffement climatique, observés également dans nos régions pourraient accélérer d'avantage le déclin de cette espèce de zones humides, en changeant significativement son environnement.

Plusieurs mesures ont été suggérées pour lutter contre les effets négatifs de la fragmentation du paysage sur les espèces végétales et mon travail relève l'importance de telles mesures. Il est essentiel d'empêcher la disparition des zones humides actuelles, les habitats naturels doivent être prioritairement protégés et toute perte en surface de zones naturelles doit être compensée de manière respectueuse. De plus, ces habitats naturels doivent être maintenus à des tailles suffisantes afin d'éviter une dérive génétique trop importante et l'accroissement de consanguinité dans les populations. Pour terminer, les zones humides doivent être connectées entre elles afin de former un réseau d'habitats adéquats permettant un flux de gènes suffisant entre les populations. Ce flux de gène peut se faire soit par le déplacement des pollinisateurs, soit par une dispersion des graines suffisamment étendue. Finalement, les mesures de restauration qui peuvent s'ajouter à cet ensemble de recommandations doivent amener, par un flux génétique artificiel, une augmentation de la diversité génétique dans les petites populations ayant un taux de consanguinité élevé.

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Curriculum vitae

Personal data

Name	Hoehn
First Name	Sophie
Born	22th September 1978
Place of origin	Les Genevez (Jura), Switzerland

Education

PhD thesis 2002-2006	**University of Zürich** Multi-year Demography and Eological Gnetics of the Cmmon Pant *Lychnis flos-cuculi* in a Fagmented Landscape
Diploma thesis 2002	**University Lausanne** Study and characterization of calling sites of the tree frog (*Hyla arborea*) in western Switzerland.
University 1998-2002	**University of Lausanne** Studies at the Faculty of Biology with specializations in Ecology, Zoology and Botanic
Maturité Fédérale 1994-1997	**Lycée Cantonal de Porrentruy** Maturité Fédérale, type langues modernes (Italien)